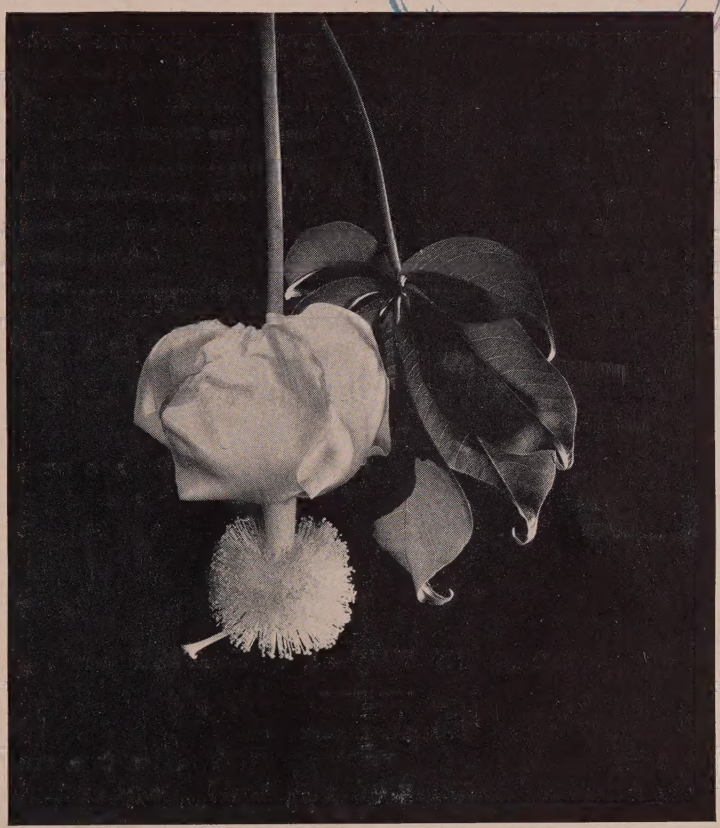
VOLUME XLIII NUMBER 2

THE HAWAIIAN PLANTERS' RECORD



The flower of the Baobab tree, (*Adansonia digitata*), an African species of which there are at least two individuals of fruiting age in Honolulu. There are many of these trees on the plains of Uganda which will exceed both in age and in diameter of trunk the famed redwoods of California.

SECOND QUARTER 1939

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THE HAWAIIAN PLANTERS' RECORD

Vol. XLIII SECOND QUARTER 1939 No. 2

A quarterly paper devoted to the sugar interests of Hawaii and issued by the Experiment Station for circulation among the plantations of the Hawaiian Sugar Planters' Association.

In This Issue:

A Modern Statistical Analysis for Field Experiments:

With a new interest being shown in the modern methods of studying the results from field experiments, it seems opportune to present a non-technical discussion, with working examples, of the "analysis of variance"—a statistical tool which may be used to measure the significance of the effects of the applied treatments. This is especially necessary since our attention has become focused on the plan of field testing in "factorial experiments," for it has made possible a reliable estimate of the results obtained from these more complex plans.

Pythium Root Rot of Sugar Cane in Louisiana:

Exhaustive studies of *Pythium* root rot of sugar cane in Louisiana during the period since 1924 are reported by R. D. Rands and Ernest Dopp in Technical Bulletin 666 of the U. S. Department of Agriculture, published in October 1938. This valuable contribution to an understanding of the root rot problem is reviewed briefly and the authors' summary is quoted in full, because of the importance of this disease to our industry when varieties are naturally susceptible or become so in a modified soil environment.

The failure of the old noble varieties in 1923-1926, in part due to *Pythium* root rot, caused a virtual collapse of the Louisiana sugar industry. Varietal introductions by the U. S. Department and cooperating agencies restored the industry to the prosperity level existing prior to 1907. Root rot is reported as being the most important factor in the root disease complex which continues to be a serious problem on these more resistant and vigorous hybrid canes.

The responsible agents in root rot are fungi of the genus *Pythium*, of which *Pythium arrhenomanes* is by far the most active. This species is identical with the *Pythium* sp. first reported in Hawaii as a cause of root rot of cane in 1919. The reported increase in virulence of *P. arrhenomanes* coincident with the general adop-

tion of resistant varieties which favored the survival of the more virulent strains, indicates that root rot is a dynamic rather than a static factor in cane culture.

Influence of Potash Fertilization Upon the Production and Composition of Dry Matter:

Studies that show effects which the stage of maturity of a crop may have upon its composition, and also the lack of a correlation between yields and their potash content, make it appear improbable that a recommendation for specific potash fertilization can safely be based upon the crop's potash-composition figures.

The Growth of Plants in Water and Sand Cultures:

Soilless agriculture and its synonyms, plant requirements, the development and use of a soluble plant fertilizer, the water- versus the sand-culture method for growing plants, and practical uses of soilless agriculture are discussed in this article. The preparation of two complete nutrient solutions and directions for their use by Hoagland and Arnon of the University of California are given for the benefit of those who may wish to prepare their own culture solutions.

Variation in Available Nutrients in an Uncropped Surface Soil:

A semi-monthly analysis, for available nutrients from an uncropped soil during a period of two years, reveals variations which indicate the limitation of soil analyses and the hazard of a strictly quantitative interpretation therefrom.

Colorimetric Method for the Determination of Sulfate in Cane Juice:

A colorimetric method for determining the amount of sulfate in cane juice is presented. The method is rapid, especially when using the permanent inorganic color standards which are described. The sulfate in the juice, which is brought to a degree of acidity represented by a pH of about 4.0, is precipitated with an excess of standard barium chloride solution. Sodium rhodizonate is added to the excess barium which forms the colored solution and the latter is compared with the standards. A sample of crusher juice can usually be analyzed to within a few per cent of its actual sulfate content in a matter of minutes. The method is applicable to other aqueous solutions containing sulfates.

The Third Study of Water and Cane Ripening:

When the amount of water in the soil reaches the wilting point, very little production of sugar can take place in the leaves of the sugar cane plant. A plentiful supply of water is essential not only for the formation of sucrose in the blades, but also to facilitate its transport to the stem and its expression in the juice.

A Modern Statistical Analysis for Field Experiments

The Analysis of Variance for Simple Factorial Experiments

By R. J. Borden

Introduction

The application of statistical measures to results from field experiments provides a safety factor which discourages attempts to definitely attribute small apparent differences, which have not been adequately measured, to the known differential treatments which have been applied. Through their use we are also able to determine the limits between which the true value of a treatment effect is most likely to occur.

The field investigator of sugar cane problems should look upon a statistical analysis simply as another technical tool for his use. It will take its proper place along with his surveying instruments, his steel tape, scales, refractometer, calculating machine, slide rule, and mathematical tables and formulae, in helping him to note and measure the factors and relationships that are involved in his investigation. It does not, however, furnish the explanation of these observations. Neither does it supply a substitute for clear-cut thinking, nor for his logical common-sense analysis that is based on knowledge he has secured from past experience, nor can it compensate in any way for an unskilled or an inadequate technique which may have been used.

When a statistical analysis has been completed, its results must be compared with the original assumptions which were set up when the objective of the investigation was proposed as a field test problem. If the analysis agrees with the assumptions, the results are clearly interpreted and should then be stated in terms which are understandable to those who will use them. If the statistical result and the hypothesis are not in agreement, both will need to be carefully re-examined—the hypothesis, to see if it was correctly deduced; the statistics, to see if they were properly and correctly worked out. Then, if the hypothesis and the results are still found to be inconsistent, the results will need to be recorded as actual observations that are not consistent with the expected results and hence subject to further study before definite conclusion is drawn.

Agronomists are in general agreement that a minimum level for judging whether measured differences in field experiments are most likely the effect of chance, (and hence not definitely attributable to known treatment differentials), is represented by a probability of 5 in 100 ($P = .05$), more commonly spoken of as odds of 19 to 1. If we use this 1-in-20 level of significance in judging our results, we must recognize that 1 out of every 20 results may be judged significant when there are no treatment effects at all. Hence we must avoid emphasis on such statistical significance when the results apparently have no consistent relationship with a well-established belief based on long experience: judgment had best be reserved until further evidence is secured.

An interpretation of non-significance does not mean that no treatment effect exists; it simply means that the observed apparent effect would be likely to show

up in excess of once in twenty results by chance alone, even if there were no real treatment effects.

Plantation men who are interested in conducting field experiments, as well as those who study their results, are being impressed with the scope and values which the modern statistical methods offer. All too few, however, who have occasion to use statistical methods are sufficiently mathematically inclined to think in mathematical terms, and most of those who wish to interpret the data themselves are inclined to look askance at all mathematical expressions and symbols. Hence, in the belief, based on personal experience, that the application of statistical measures to the results of field experiments can best be learned by actual practice in their application, we have prepared the examples which follow with complete work sheets so that he who is interested may follow the mechanics of the mathematical procedures which are used.

It is more than likely that these plans which we have discussed in the examples that follow will be criticized by the strict adherents to a policy of randomization for plot positions in experimental areas. Better men than we have argued the pros and cons of randomization. For our part, we have convinced ourselves that more reliable yields from plots carrying a mature sugar cane crop can be secured when such plots have had an intelligent arrangement than when they have been assigned their position through randomization. "Student,"* after looking over our designs for balanced arrangements and the results of many studies we have made from our Blank Test data, once wrote us that "your results are just what I should have excpected, that is to say, that the balanced arrangement is better than the random," and again, "I am quite sure that there is much to be gained by a balanced arrangement."

There are few who question our contention that the cane yields will be more reliable from a balanced than from a random plot arrangement, but there are many who contend that no valid estimate of error can be made for such yields because the plot arrangement has not been at random. Student has commented that "all these people mean by valid is that they are rigidly justified in using the various (statistical) tables, and that is an advantage which is not only not worth going far to get but may often be dearly bought. . . . What is claimed, is that if a randomized layout is used, the various tables, Z, T, etc., will give you the probability of obtaining eccentric results, but this is only true *before* the actual layout is selected from among the possible random arrangements. *After* the particular layout is chosen, the tables no longer apply strictly, and this can be seen from the fact that the regular arrangements are among those which could be drawn at random. It is not, unfortunately, possible to estimate the probability accurately in any but a random arrangement, but that does not prevent your being able to say that a balanced arrangement, for example, will give results which are more accurate than those which would have been obtained with a random arrangement."

The fact, as Student points out, that a balanced arrangement *may* actually have been drawn at random, and that if it had been so drawn, one would be correct in using the statistical tables and methods which would be denied if the balanced arrangement was deliberately planned, fails to make common sense for most agronomists. The further fact, that this tossing of a coin or drawing from a hat to decide the plot arrangement, may actually place several plots of the same treat-

* The late W. S. Gosset in personal communication.

ment adjacent and coinciding with an abnormally high-or-low-fertility spot, or at such a position within the field test area where one knows before the test is started that a biased result will be secured, does not elicit much support from a practical field man who knows the conditions under which field testing must be done. Furthermore, the fact that most agronomists have quite arbitrarily set odds of 19 to 1 ($P = .05$) as their minimum level of significance makes it appear unreasonable to insist on a blind and rigid adherence to precise mathematics, for we would accept odds of 20 to 1 as significant and consider odds of 18 to 1 as not significant in our final interpretation. Hence, unless our experimental plans will give us yields with as little error as possible, that is, yields which are free from bias, then any measure of significance is apt to give a false degree of confidence in the results. Surely then it is more reasonable to err, if by chance we err at all, in the direction of *estimating* the significance of a result than in actually obtaining such result; the really important large differences between field treatments will be detected without a highly precise mathematical tool.

We might argue this issue still further but we have probably said enough to indicate that we feel satisfied that the ordinary statistical tables can be intelligently used to study data from balanced arrangements. The required randomness for plot positions is most likely secured when the decision is made to locate the first plot of a plan, which has been drawn up in the office, at a certain point in the field, since the heterogeneous nature of soil fertility in our cane lands provides thereafter the important features of a random distribution.

The Analysis of Variance

Although R. A. Fisher, of Rothamsted, introduced his method of analysis of variance some 15 years ago, it has only been in more recent years that our American agronomists have quite generally adopted it for studying the results of their experiments.

As applied to field experiments, the analysis of variance first separates the total variation of the experiment into two parts—one identified with the differences between the plot yields which have received the same treatment (variation *within* treatments), and the other identified with the variation among the means of the different treatments (variation *between* treatments). After this separation has been made, we are able to test whether the treatment means are significantly different, that is, whether they vary more than would be expected by chance alone. For example, we may take the following yields (T.C.A.) from 4 replications of each of 3 treatments tested on 12 plots:

T.C.A. FROM 3 TREATMENTS (A, B, X)

	A	B	X	Total
	62	64	54	
	68	60	60	
	62	68	52	
	66	72	50	
Sums	258	264	216	738
Means	64.5	66.0	54.0	61.5

The total variation in this experiment may be represented by the sum of all of the squared deviations $(d)^2$ of each plot yield from the mean yield of all plots (61.5), e.g.

A		B		X		Total variation
d	d^2	d	d^2	d	d^2	
+ .5	.25	+ 2.5	6.25	— 7.5	56.25	
+ 6.5	42.25	— 1.5	2.25	— 1.5	2.25	
+ .5	.25	+ 6.5	42.25	— 9.5	90.25	
+ 4.5	20.25	+ 10.5	110.25	— 11.5	132.25	
						505.00

Note: The sum of the + and — figures in the "d" columns should equal zero (in this example, we have + 31.5 and — 31.5).

The amount of variation, which exists between the plot yields which have received the same treatment, is then found by summing the squared deviations of each plot yield from the mean yield of its respective treatment ("A" at 64.5, "B" at 66.0; "X" at 54.0), e.g.

A		B		X		Total variation within treatments
d	d^2	d	d^2	d	d^2	
— 2.5	6.25	— 2.0	4.0	0	0	
+ 3.5	12.25	— 6.0	36.0	+ 6.0	36.0	
— 2.5	6.25	+ 2.0	4.0	— 2.0	4.0	
+ 1.5	2.25	+ 6.0	36.0	— 4.0	16.0	
						163.00

This pooled sum of the squared deviations (163) represents an uncontrolled variation *within* all of the treatments (often called the experimental error) and when subtracted from the total sum of the squares (505) it gives the sum of the squares which indicates the variation *between* the treatments: 505 — 163 or 342. This sum of the squares *between* treatments (342) may also be secured by squaring the deviation of each treatment mean from the general mean (61.5), summing these squared deviations, and multiplying this sum by 4 (since there are 4 plots represented in each treatment mean), e.g.

Treatment		Mean yield	d	d^2
A	=	64.5	+ 3.0	9.00
B	=	66.0	+ 4.5	20.25
X	=	54.0	— 7.5	56.25
General mean		61.5		85.50
				× 4
				342

Any sum of squares divided by its respective number of degrees of freedom* gives the variance. The degrees of freedom for our example are as follows:

Between treatments	2
Within treatments (3+3+3)	9
Total	11

* Degrees of freedom: Equivalent to n — 1, i.e., one less than the number of observations concerned in the particular sum-squares calculation.

Thus the total variance would be 505 ÷ 11 or 45.9, and the variance *between* treatments (i.e., due to the treatment differences) is 342 ÷ 2 or 171. Similarly the variance *within* treatments (i.e., due to position and to the unidentified error) is 163 ÷ 9 or 18.1.

However, it is upon the ratio of the *mean* variance *between* treatments to the *mean* variance *within* treatments that the test of significance is based. Snedecor has given us mathematical tables for determining whether this ratio, $\frac{\text{mean variance between treatments}}{\text{mean variance within treatments}}$ which he calls "F," is significant.

So we set up the foregoing facts for the analysis of variance as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square or variance	"F" Found(1)	"F" Required(2)
Between treatments	2	342	171	9.4	4.26
Within treatments	9	163	18.1		
Total	11	505			

(1) $\frac{171.0}{18.1} = 9.4$

(2) A figure obtained from Snedecor's tables, for a probability of .05 or odds of 19 to 1, and the proper number of degrees of freedom.

Therefore, since "F" as found is greater than "F" required, this analysis would indicate that the mean variance between or for treatments is significant, i.e., it would not be expected to occur by chance more often than once in twenty similar tests.

Mathematical calculations concerned with analysis of variance may be greatly simplified by using a shortened method and working with totals rather than averages, and hereafter in our examples we shall use the short methods. The procedure used in the short method for finding the sum squares from the example just cited would be as follows (using same data):

A		B		X		Total Y^2
Y	Y^2	Y	Y^2	Y	Y^2	(all treatments)
62	3844	64	4096	54	2916	
68	4624	60	3600	60	3600	
62	3844	68	4624	52	2704	
66	4356	72	5184	50	2500	
Sums 258		264		216		45892

Total (T) $258 + 264 + 216 = 738$.

$$\text{Correction factor } \frac{T^2}{n} = \frac{(738)^2}{12} = \frac{544644}{12} = 45387$$

*Total Sum-Squares:**

$$\begin{array}{r} 45892 \\ -45387 \\ \hline \end{array}$$

Corrected Total Sum-Squares 505 (with 11 degrees of freedom)

* This term "Sum-Squares" always refers to the sum of the squares of the deviations from the mean yield of all plots.

Sum-Squares Treatments:

$$\frac{(258)^2 + (264)^2 + (216)^2}{4} = \frac{182916}{4} = 45729$$

$$-45387$$

Corrected Sum-Squares Treatments 342 (with 2 degrees of freedom)

*Sum-Squares Error** (i.e., within treatments):

$505 - 342 = 163$ (with 9 degrees of freedom)

These figures are then set up for further examination in the Analysis of Variance Table as previously shown on page 77.

To better understand the "Analysis of Variance," one needs to be aware of the following relationships:

1. That the Total Sum-Squares is made up of the Sum-Squares *between* the Treatments and the Sum-Squares *within* the Treatments; furthermore, that the Sum-Squares *within* Treatments is made up of the Sum-Squares due to Position (Blocks, Rows and Columns, Squares) and to the Sum-Squares due to Error. (In complex or factorial experiments it will also include the sum of the squares due to the interactions of the various factors.)

2. That the Total Sum-Squares for an experiment is likewise made up of the Sum-Squares *between* the Blocks (or Rows and Columns in a Latin-square layout) and the Sum-Squares *within* the Blocks; furthermore, that the Sum-Squares within Blocks is made up of the Sum-Squares for Treatments and for Error.

3. That the Sum-Squares for position may be identified and determined, (a) for Blocks (in a block arrangement); (b) for Rows and Columns (in a single Latin square); and (c) for Rows, Columns, and Squares (in a multiple Latin-square layout).

4. That the Sum-Squares for the combined Treatments in a factorial experiment (which we shall discuss later) is made up of the Sum-Squares for each of the separate factors and the Sum-Squares of their Interactions.

5. That the respective mean Variances are found by dividing their Sum-Squares by their number of degrees of freedom $(n - 1)$.

6. That the measures of significance (odds, "F" values, etc.) are based on the relation between the mean variance *between* Treatments and the mean variance *within* Treatments, i.e., $\frac{\text{Mean variance between treatments}}{\text{Mean variance within treatments}} = \text{measure of significance.}$

If the extent of certain contributing factors to the variation *within* treatments can be identified, its magnitude can be reduced and the statistical measurement becomes more refined. Thus, when certain layouts or arrangements of plots have been used, a positional effect (from blocks, or from rows and columns, or from squares) can be determined and subtracted; in factorial experiments when treatments have been combined, an effect of interaction can be measured and deducted from this variation within the treatments. After such deduction, the unidentified

* The Sum-Squares within the Treatments is convertible into the Sum-Squares for Error if its component parts are not still further identified.

remainder is the only part which is due to error, and our fraction or ratio for measuring the effect of treatment then becomes $\frac{\text{Variance between treatment}}{\text{Variance for error}}$

For example, we might have these data from a test on 30 plots carrying 10 replicates of 3 treatments:

Total Sum-Squares for 30 plots = 400, with 29 degrees of freedom.
Sum-Squares between 3 treatments = 60, with 2 degrees of freedom.
Sum-Squares within 3 treatments = 340, with 27 degrees of freedom.

The mean variance for treatments is then $\frac{60}{2}$ or 30, while the mean variance within Treatments (the error) is $\frac{340}{27}$ or 12.6.

The "F" value found would then be $\frac{30}{12.6}$ or 2.4 and as this is less than "F" required (3.35) for minimum significance, we would have to conclude that the treatment effect may have been due to chance.

On the other hand, if the test has been laid out so that some effect of the positional variation can be determined, and let us assume that we have found a Sum-Squares for 10 Blocks (each Block carrying one plot each of all 3 treatments) amounting to 240, our Sum-Squares within Treatments (340) can be reduced by 240, and the Sum-Squares for Error is thereby only 100; then with 27 minus 9 or 18 degrees of freedom left for Error (because the Blocks have accounted for 9 degrees of freedom), the mean variance for Error becomes $100 \div 18$ or 5.6. The "F" value now becomes $\frac{30}{5.6}$ or 5.36, which is more than the "F" required (3.55) for significance, and hence gives a fair degree of assurance that there has been a real effect of treatment.

So let us examine and explain two completed examples, one which illustrates the steps in using this statistical measure on yields obtained from a plot arrangement in Blocks, and the other from an arrangement in a double Latin square:

Our first example is with 2 treatments (A, X), balanced, in 8 blocks (I to VIII) using 16 plots.

Arranged as follows:

YIELDS AS T.C.A.

I	A	76	X	70	V
	X	74	A	72	
II	X	79	A	77	VI
	A	81	X	83	
III	A	73	X	80	VII
	X	73	A	82	
IV	X	71	A	74	VIII
	A	76	X	70	

DETAIL FOR THE ANALYSIS OF VARIANCE

Source	Degrees of freedom
Blocks	7
Treatments	1
Error	7
Total	15

The *Total Sum* of the *Squares* of the deviations of each plot yield from the average yield of all the plots includes all of those factors (treatment, position, unknowns) that make one plot yield differently from another.

This *total sum-squares* is most easily obtained by squaring each plot yield, summing these squares, and deducting the proper correction factor (which is the squared sum of all plot yields divided by the number of plots represented in this sum). For example:

$$\text{Correction factor } (76 + 74 + 79 + 81 + \ldots 82 + 74 + 70)^2 = \frac{(1211)^2}{16} = 91658$$

$$\text{Then } (76)^2 + (74)^2 + (79)^2 + \ldots (82)^2 + (74)^2 + (70)^2 = 91931$$

Minus the correction factor 91658

Corrected Total Sum-Squares 273 (with 15 degrees of freedom)

This *Total Sum-Squares* is made up of the variation *between* the Blocks and the variation *within* the Blocks.

We next obtain the amount of variation which is contributed by the position of the 8 Blocks on the experimental area. Since each Block is similar, in that it contains one plot of both treatments, we determine the Block totals:

Block I	$76 + 74 = 150$	Block V	$70 + 72 = 142$
Block II	$79 + 81 = 160$	Block VI	$77 + 83 = 160$
Block III	$73 + 73 = 146$	Block VII	$80 + 82 = 162$
Block IV	$71 + 76 = 147$	Block VIII	$74 + 70 = 144$

These Block totals are then squared, the squared totals summed, and the sum divided by the number of individual plot yields which were represented in each Block total used. The correction factor is then deducted and the result is the amount of the Total Sum-Squares that can be allocated to the Block's position, i.e., the amount between Blocks. For example:

$$\frac{(150)^2 + (160)^2 + (146)^2 + \ldots (162)^2 + (144)^2}{2} = \frac{183769}{2} = 91885$$

$$-91658$$

Corrected Sum-Squares between Blocks 227 (with 7 degrees of freedom)

The difference between the Total Sum-Squares and the Sum-Squares *between* Blocks is the Sum-Squares *within* the Blocks; this would be *273 — 227* or 46. This Sum-Squares *within* Blocks is made up of variation which is due to the different treatments and to the undeterminable effects which we call "Error."

The Sum-Squares for Treatments can be determined by squaring the treatment totals, dividing by the number of plot yields represented in each treatment total, and subtracting the correction factor. For example:

Treatment "A" totals: $76 + 81 + 73 + 76 + 72 + 77 + 82 + 74 = 611$ (Avg. 76.4 T.C.A.)
Treatment "X" totals: $74 + 79 + 73 + 71 + 70 + 83 + 80 + 70 = 600$ (Avg. 75.0 T.C.A.)

$$\text{Then: } \frac{(611)^2 + (600)^2}{8} = \frac{733521}{8} = 91665$$

$$-91658$$

Corrected Sum-Squares for Treatments 7 (with 1 degree of freedom)

The difference between the Sum-Squares within the Blocks and this Sum-Squares for Treatments is the Sum-Squares for Error, upon which our test of significance is to be based. Hence 46 — 7 or 39 is the Sum-Squares for Error in this experiment (and this item will have the remaining or 7 degrees of freedom).

It is now necessary to determine the mean variance for Treatment and for Error, because it is from the ratio of these two mean variance figures that we secure our estimate of the effect of the treatment. These mean variances are determined by dividing the proper Sum-Squares by their respective "degrees of freedom." Thus we have:

For Treatment: $7 \div 1$ or 7.0 as the mean Treatment variance, and
For Error: $39 \div 7$ or 5.57 as the mean Error variance,
and the ratio of Treatment variance to Error variance is $\frac{7.0}{5.57}$ or 1.26.

Reference to Snedecor's tables for "F" will indicate that this value (1.26) is far below the required value (5.59) which agronomists will accept as satisfactory evidence of an effect of treatment. Hence the differences found between the Treatment totals are considered as quite apt to be due to chance alone, and we record them as "not significant."

Our second example is with 3 treatments (A, B, C) arranged in 2 Latin squares, and using 18 plots.

Arranged as follows:

	SQUARE I			SQUARE II		
	Column 1	Column 2	Column 3	Column 1	Column 2	Column 3
Row 1	A 80	B 84	C 86	B 84	C 96	A 78
Row 2	C 89	A 84	B 78	A 77	B 83	C 94
Row 3	B 88	C 92	A 76	C 97	A 80	B 89

Details of Work Sheet:

SQUARE I

Row totals	Column totals	Treatment totals
$80 + 84 + 86 = 250$	$80 + 89 + 88 = 257$	"A" $80 + 84 + 76 = 240$
$89 + 84 + 78 = 251$	$84 + 84 + 92 = 260$	"B" $84 + 78 + 88 = 250$
$88 + 92 + 76 = 256$	$86 + 78 + 76 = 240$	"C" $86 + 89 + 92 = 267$
Square totals 757	757	757

SQUARE II

Row totals	Column totals	Treatment totals
$84 + 96 + 78 = 258$	$84 + 77 + 97 = 258$	"A" $78 + 77 + 80 = 235$
$77 + 83 + 94 = 254$	$96 + 83 + 80 = 259$	"B" $84 + 83 + 89 = 256$
$97 + 80 + 89 = 266$	$78 + 94 + 89 = 261$	"C" $96 + 94 + 97 = 287$
Square totals 778	778	778

Correction factor: $\frac{(757)^2}{9} = 63672$ (for Square I only)

Correction factor: $\frac{(778)^2}{9} = 67254$ (for Square II only)

Correction factor: $\frac{(757+778)^2}{18} = \frac{(1535)^2}{18} = 130901$ (for Totals)

Total Sum-Squares:

$(80)^2 + (89)^2 + (88)^2 + (84)^2 + \ldots (94)^2 + (89)^2 = 131637$

-130901

Corrected Total Sum-Squares 736 (with 17 degrees of freedom)

Sum-Squares for Rows in Square I:

$\frac{(250)^2 + (251)^2 + (256)^2}{3} = \frac{191037}{3} = 63679$

-63672

Corrected 7 (with 2 degrees of freedom)

Sum-Squares for Rows in Square II:

$\frac{(258)^2 + (254)^2 + (266)^2}{3} = \frac{201836}{3} = 67279$

-67254

Corrected 25 (with 2 degrees of freedom)

Corrected Sum-Squares for rows in both squares:
$7 + 25 = 32$ (with $2 + 2$ or 4 degrees of freedom)

Sum-Squares for Columns in Square I:

$\frac{(257)^2 + (260)^2 + (240)^2}{3} = \frac{191249}{3} = 63750$

-63672

Corrected 78 (with 2 degrees of freedom)

Sum-Squares for Columns in Square II:

$\frac{(258)^2 + (259)^2 + (261)^2}{3} = \frac{201766}{3} = 67255$

-67254

Corrected 1 (with 2 degrees of freedom)

Corrected Sum-Squares for columns in both squares:
$78 + 1 = 79$ (with $2 + 2$ or 4 degrees of freedom)

Sum-Squares for Two Squares:

$\frac{(757)^2 + (778)^2}{9} = \frac{1178333}{9} = 130926$

-130901

Corrected Sum-Squares Squares 25 (with 1 degree of freedom)

Sum-Squares for Treatments and Squares:

$\frac{(240)^2+(250)^2+(267)^2+(235)^2+(256)^2+(287)^2}{3} - = \frac{394519}{3} = 131506$

-130901

Corrected 605 (with 5 degrees of freedom)

Sum-Squares for Treatments A, B, C:

$$\frac{(240+235)^2+(250+256)^2+(267+287)^2}{6}=$$

$$\frac{(475)^2+(506)^2+(554)^2}{6}=\frac{788577}{6}=\ 131430$$

$$-130901$$

Corrected 529 (with 2 degrees of freedom)

Sum-Squares for Interaction (between Treatments and Squares):

$605-(25+529)=51$ [with $5-(1+2)$ or 2 degrees of freedom]

Sum-Squares for Error:

$736-(32+79+25+529+51)=$
20 [with $17-(4+4+1+2+2)$ or 4 degrees of freedom]

And from the foregoing, we can set up a table for the analysis of variance, as follows:

Source of variation	Degrees of freedom	Sum of squares	Mean square or variance
Rows	(2+2) 4	32	8.0
Columns	(2+2) 4	79	19.8
Squares	1	25	25.0
Treatments	2	529	264.5
Interaction (Treatment and Squares)*	2	51	25.5
Error	4	20	5.0
Total	17	736	

Our chief interest is now in the ratio of this treatment variance (264.5) to the error variance (5.0). Since their ratio ("F") of $\frac{264.5}{5.0}$ equals 52.9 and the required "F" for significance (from Snedecor's tables) is only 6.94, we have a good indication that the treatments have been effective.

Since the analysis has indicated a definite effect of treatment, we can now calculate the significance of the differences between the individual treatment totals.

The standard deviation for the experiment is the square root of the error variance, i.e., $\sqrt{5.0}$ or 2.24. The standard error *for the total* of 6 plots of any one treatment is $\sqrt{5.0\times 6}$ or 5.48, and a difference between any two such totals greater than $t\dagger\sqrt{5.0\times 6\times 2}$ or $2.78\dagger\sqrt{60.0}$ which is 21.5 tons is significant. Treatment totals are:

"A" = 475 tons "B" = 506 tons "C" = 554 tons

* Note: Sometimes one finds a significant interaction between Treatments and Squares, that is, there may be a difference in the effectiveness of the treatments when tested on different parts of the experimental area. In the present example, this type of interaction is probably not in effect since the ratio of the Interaction variance to the Error variance $\frac{25.5}{5.0}$ or 5.1 is less than the required "F" (6.94) which is needed for significance, thus indicating that this particular interaction effect might quite easily be due to chance. When such an interaction is not significant, it may be advisable to put its associated sum-squares and degrees of freedom back with error, to afford a more conservative estimate of the treatment effects.

† The values of "t" for the number of degrees of freedom associated with the error variance are obtainable from Fisher's tables of "t" values.

Hence both treatments "B" and "C" are distinctly better than "A," and "C" is also better than "B." If desirable, these treatment totals can now be expressed as averages by dividing by the number of plots represented: thus the "A" treatment average would be 79.2 tons, the "B" average is 84.3 tons, and "C" averages 92.3 tons.

From the foregoing discussions it should be clear just how the precision by which the effect of treatment can be measured is increased when the variation within treatments can be broken down into the effects of position and the effect of error. But this assumes that the plot arrangement has been of such a nature that the amount of this positional variance can be determined. So if we are to use this method of statistical analysis, we must be certain that our experimental layout has been planned to allow for the best identification of its positional variance.

For reference, and as acceptable examples for our "Grade A" sugar cane experiments, we offer the 4 following plans (Figs. 1 to 4).

PLANS FOR ANALYSIS OF VARIANCE

For any 3 treatments (A, B, X) using a total of 27 plots

IN MULTIPLE LATIN SQUARES (I II III)

I	A	B	X	A	X	B	III
	B	X	A	X	B	A	
	X	A	B	B	A	X	
II	X	B	A				
	B	A	X				
	A	X	B				

Fig. 1

DETAIL FOR THE ANALYSIS OF VARIANCE

Source	Degrees of freedom
Rows (2+2+2) = ...	6
Columns (2+2+2) = ...	6
Squares	2
Treatments	2
Interaction* (Treatments & Squares)	4
Error	6
Total	26

* See footnote on page 83.

IN BALANCED ARRANGEMENT IN 9 BLOCKS (I to IX)

	V				
I	A	X	A	VIII	
	B	B	B		
	X	A	X		
II	X	A	X	IX	
	B	B	B		
	A	X	A		
III	A	X			
	B	B			
	X	A			
	VII				
IV	X				
	B				
	A				

Fig. 2

DETAIL FOR THE ANALYSIS OF VARIANCE

Source	Degrees of freedom
Blocks	8
Treatments	2
Error	16
Total	26

For any 4 Treatments (A, B, C, D) using a total of 32 plots

IN DOUBLE LATIN-SQUARE ARRANGEMENT

I	A	D	B	C
	B	C	A	D
	C	B	D	A
	D	A	C	B
II	D	B	C	A
	A	C	B	D
	B	D	A	C
	C	A	D	B

Fig. 3

DETAIL FOR THE ANALYSIS OF VARIANCE

Source	Degrees of freedom
Rows (3+3)	6
Columns (3+3)	6
Squares	1
Treatments	3
Interaction (Squares & Treatments)	3
Error	12
Total	31

IN BALANCED ARRANGEMENT IN 8 BLOCKS

	IV			
I	A	D	A	VII
	B	C	B	
	C	B	C	
	D	A	D	
II	D	A	D	VIII
	C	B	C	
	B	C	B	
	A	D	A	
III	A	D		
	B	C		
	C	B		
	D	A		
	VI			

Fig. 4

DETAIL FOR THE ANALYSIS OF VARIANCE

Source	Degrees of freedom
Blocks	7
Treatments	3
Error	21
Total	31

Factorial Experiments for Sugar Cane

Ordinarily it has been our custom to install separate experiments to test separate issues. Thus we have had our "Amounts-of-Nitrogen" (AN) tests, and our "Amounts-of-Potash" (AK) tests; our "Variety" tests, and our "Cultivation" tests. In these AN tests, we have arbitrarily set a uniform total for the potash (and phosphate) applications at what we *believe* to be an adequate amount to insure its not being another limiting factor; in the AK tests we have specified a definite amount of nitrogen and phosphate which was to be used. Similarly, in our variety and in our cultivation tests we have quite arbitrarily set the level of fertilization.

It is not unlikely that the response to varied amounts of one nutrient may be different when tested at different levels of another nutrient. Thus, conclusions on the optimum amount may not be safely applied in a field practice that differed in its amounts of the other nutrients from those under which such optimum was determined. And it is quite possible that variety superiority may be indicated at one level of fertilization and may not be noted at some other level.

Thus there is a growing interest in the factorial type of field experiment in which two or more factors are compared in all possible combinations of their several different levels. Results are thus obtained on the response from the different factors singly, and at the same time upon their respective interactions, i.e., the way in which a change in one factor is influenced by a change in another.

Even though such interactions do not actually occur, and the response to one factor is substantially the same for all variations of another factor, the factorial experiment makes it possible to use a greater number of plots in making estimates of the effect of the different factors, and this greatly increases the precision with which the results can be measured. For instance, with 3 amounts of nitrogen and 3 amounts of potash, there are 9 possible treatment-combinations. If we identify the 3 nitrogen levels as N1, N2, and N3, and the 3 potash levels as K1, K2, and K3, these 9 treatment combinations will be:

N1 K1	N1 K2	N1 K3
N2 K1	N2 K2	N2 K3
N3 K1	N3 K2	N3 K3

With 6 replications for these 9 combinations, from an area with 54 plots, we could have a mean yield for each nitrogen level as secured from 18 plots, and also a mean yield for each potash level from 18 plots. This is possible because the potash differentials occur an equal number of times with each nitrogen level, and similarly, the nitrogen differentials occur an equal number of times with each potash level. If two separate tests had been installed on these 54 plots (27 plots for each test) then our mean yields would have come from only 9 plots for each level of N and of K. Thus this factorial plan would give us doubled precision for determining the effect of the 3 levels of both N and of K.

This comparative precision in estimating the effects of treatments in separate vs. factorial experiments may also be illustrated as follows: Our minimum "Grade A" standards call for testing (a) varieties with 5 replicates, and (b) fertilizer treatments with 7 replicates. Hence to test 2 varieties and 2 fertilizer treatments in separate experiments we need at least 24 plots. If we use these same 24 plots for a factorial experiment, combining both varieties with both fertilizers, we can have 12 plot yields to average for the effect *of each factor* (variety and fertilizer), and the estimate of the experimental error will be based on more than double the number of degrees of freedom obtainable in either single test.

Without further discussion of the principles involved in factorial designs, we give a few simple examples, using the analysis of variance therewith:

Example No. 1

A factorial experiment for 2 factors (X and Y) each at 2 levels (1 and 2); therefore, 4 combined Treatments in a balanced arrangement in 7 Blocks carrying a total of 28 plots.

Plot identity	Treatment combination	Assumed factors and amounts
A	X1 Y1	(125 lb N, 100 lb K_2O)
B	X1 Y2	(125 lb N, 200 lb K_2O)
C	X2 Y1	(175 lb N, 100 lb K_2O)
D	X2 Y2	(175 lb N, 200 lb K_2O)

PLOT ARRANGEMENT AND YIELDS (T.C.A.)

I	A 70	C 79		
	B 74	D 79		
II	D 86	B 81		
	C 74	A 70		
III	C 80	A 78		
	D 82	B 75		
IV	B 85	D 88		
	A 88	C 80		
V	A 79	C 74		
	B 76	D 80		
VI	D 83	B 76		
	C 71	A 79		
VII	C 72	A 84		
	D 79	B 72		

SET-UP FOR TOTALS

Block No.	A	B	C	D	Block totals
I	70	74	79	79	302
II	70	81	74	86	311
III	78	75	80	82	315
IV	88	85	80	88	341
V	79	76	74	80	309
VI	79	76	71	83	309
VII	84	72	72	79	307
Treatment totals	548	539	530	577	2194

Nitrogen totals:

125 lb (A + B) = 548 + 539 = 1087

175 lb (C + D) = 530 + 577 = 1107

Potash totals:

100 lb (A + C) = 548 + 530 = 1078

200 lb (B + D) = 539 + 577 = 1116

Work Sheet for Example No. 1:

Correction factor: $\frac{(2194)^2}{28} = 171916$

Total Sum-Squares:

$(70)^2 + (70)^2 + (78)^2 + (88)^2 + \ldots (83)^2 + (79)^2 =$ 172642

-171916

Corrected 726 (with 27 degrees of freedom)

Sum-Squares for Blocks:

$\frac{(302)^2 + (311)^2 + (315)^2 + \ldots (307)^2}{4} - \frac{688642}{4} =$ 172161

-171916

Corrected 245 (with 6 degrees of freedom)

Sum-Squares for Treatments:

$$\frac{(548)^2 + (539)^2 + (530)^2 + (577)^2}{7} = \frac{1204654}{7} = 172093$$

$$-171916$$

Corrected 177 (with 3 degrees of freedom)

Sum-Squares for Nitrogen:

$$\frac{(1087)^2 + (1107)^2}{14} = \frac{2407018}{14} = 171930$$

$$-171916$$

Corrected 14 (with 1 degree of freedom)

Sum-Squares for Potash:

$$\frac{(1078)^2 + (1116)^2}{14} = \frac{2407540}{14} = 171967$$

$$-171916$$

Corrected 51 (with 1 degree of freedom)

Sum-Squares for Interaction (between N and K):

$177 - (14 + 51) = 112$ (with 1 degree of freedom)

Sum-Squares for Error:

$726 - (245 + 14 + 51 + 112) = 304$ (with 18 degrees of freedom)

From these data, we then set up the pertinent items in a table for the analysis of the variance as follows:

Source of variation	Degrees of freedom	Sum squares	Mean square or variance	"F" Found	"F" Required	Remarks
Blocks	6	245	....	....	....	....
Nitrogen	1	14	14.0	.83	4.41	Not significant
Potash	1	51	51.0	3.02	4.41	Not significant
Interaction (Nitrogen and Potash)	1	112	112.0	6.63	4.41	Significant
Error	18	304	16.9	....	....	....
Total	27	726				

The "F" required to indicate a significant effect for either factor or their interaction is 4.41. Since the "F" value as found for nitrogen is only $\frac{14.0}{16.9}$ or .83, and that for potash only $\frac{51.0}{16.9}$ or 3.02, it would appear that the separate effects of nitrogen and potash are not significant. But there is a definite effect from their interaction $\frac{112.0}{16.9} = 6.63$, so the Treatment totals will need further study.

A difference between the combined treatment totals of

$t\sqrt{\text{Error Mean Square} \times n \times 2}$ or $2.10\sqrt{16.9 \times 7 \times 2}$, which amounts to 32.3 tons would be a significant amount. Hence we note these comparisons between the combined treatment totals:

"A" over "B": 548 — 539 = 9 tons Not significant
"A" over "C": 548 — 530 = 18 tons Not significant
"B" over "C": 539 — 530 = 9 tons Not significant
"D" over "A": 577 — 548 = 29 tons Not significant
"D" over "B": 577 — 539 = 38 tons (Avg. 5.43 T.C.A.) Significant
"D" over "C": 577 — 530 = 47 tons (Avg. 6.71 T.C.A.) Significant

Our interpretation of the results then becomes: (a) That there is a definite yield increase for 175 lbs. of nitrogen over 125 lbs. when potash is supplied at 200 lbs. ("D" over "B"), but not when potash is at only 100 lbs. ("C" over "A"); and (b) that there is also a gain for 200 lbs. of K_2O over 100 lbs. when nitrogen is supplied at 175 lbs. ("D" over "C") but not when only 125 lbs. of N is given ("B" over "A").

Thus it is apparent that the factorial plan has made it possible to show real effects from nitrogen and potash applications which might have been lost had the issues been tested in separate experiments.

Example No. 2

Our second example is another 2×2 Factorial Experiment for 2 Factors each at 2 levels; therefore, with 4 combined treatments arranged on 32 plots in 2 Latin Squares:

2 Factors = P and K
2 Levels = 0 and 200 lbs.

	Plot identity	Amounts lb P_2O_5	Amounts lb K_2O
Therefore 4 combinations, identified as.....	N =	0	0
	NP =	200	0
	NK =	0	200
	NPK =	200	200

Plot numbers and identities, with yields as T.C.A.

Square I								Square II			
1 NK 74	2 NPK 80	9 N 79	10 NP 71	17 NPK 78	18 NK 72	25 NP 77	26 N 71
3 N 69	4 NP 73	11 NK 78	12 NPK 84	19 NP 75	20 N 79	27 NPK 86	28 NK 76
5 NP 71	6 N 67	13 NPK 82	14 NK 74	21 N 73	22 NP 81	29 NK 84	30 NPK 82
7 NPK 76	8 NK 76	15 NP 69	16 N 77	23 NK 78	24 NPK 80	31 N 73	32 NP 75

The complete work sheet leading up to the analysis of variance is offered without further explanations:

Work Sheet for Example No. 2:

	Treatments				Position		
Square	NK	NPK	N	NP	Row totals	Column totals	Square totals
I	74	80	69	73	304	290	
	76	76	67	71	304	296	
	78	84	79	71	294	308	
	74	82	77	69	298	306	
Total	302	322	292	284	1200	1200	1200
II	72	78	79	75	298	304	
	78	80	73	81	316	312	
	76	86	71	77	320	320	
	84	82	73	75	306	304	
Total	310	326	296	308	1240	1240	1240
Treatment totals ..	612	648	588	592	Grand total		2440

Phosphate totals: "0"=612+588=1200
"200"=648+592=1240

Potash totals: "0"=588+592=1180
"200"=612+648=1260

Correction factors:

For Total Sum-Squares: $\frac{(2440)^2}{32} = 186050$

For Square I only: $\frac{(1200)^2}{16} = 90000$

For Square II only: $\frac{(1240)^2}{16} = 96100$

Total Sum-Squares:

$(74)^2 + (76)^2 + (78)^2 + (74)^2 + (72)^2 + \ldots (77)^2 + (75)^2 = 186744$

-186050

Corrected 694

Sum-Squares for Treatment totals:

$\frac{(612)^2 + (648)^2 + (588)^2 + (592)^2}{8} = \frac{1490656}{8} = 186332$

-186050

Corrected 282

Sum-Squares for Phosphate only:

$\frac{(1200)^2 + (1240)^2}{16} = \frac{2977600}{16} = 186100$

-186050

Corrected 50

Sum-Squares for Potash only:

$\frac{(1260)^2 + (1180)^2}{16} = \frac{2980000}{16} = 186250$

-186050

Corrected 200

Sum-Squares for Interaction of Phosphate and Potash:

$282 - (50 + 200) = 32$

Sum-Squares for Rows:

In Square I: $\frac{(304)^2 + (304)^2 + (294)^2 + (298)^2}{4} = \frac{360072}{4} = 90018$

-90000

Corrected 18

In Square II: $\frac{(298)^2 + (316)^2 + (320)^2 + (306)^2}{4} = \frac{384696}{4} = 96174$

-96100

Corrected 74

Total Sum-Squares Rows: $18 + 74 = 92$

Sum-Squares for Columns:

In Square I: $\frac{(290)^2 + (296)^2 + (308)^2 + (306)^2}{4} = \frac{360216}{4} = 90054$

-90000

Corrected 54

In Square II: $\frac{(304)^2 + (312)^2 + (320)^2 + (304)^2}{4} = \frac{384576}{4} = 96144$

-96100

Corrected 44

Total Sum-Squares Columns: $54 + 44 = 98$

Sum-Squares for Squares:

$$\frac{(1200)^2 + (1240)^2}{16} = \frac{2777600}{16} = 186100$$

$$-186050$$

$$\text{Corrected} \quad 50$$

Sum-Squares for Treatments in Two Squares:

$$\frac{(302)^2 + (310)^2 + (322)^2 + (326)^2 + (292)^2 + (296)^2 + (284)^2 + (308)^2}{4} =$$

$$\frac{745664}{4} = 186416$$

$$-186050$$

$$\text{Corrected} \quad 366$$

Sum-Squares for Interaction (Treatments and Squares):

$$366 - (50 + 282) = 34$$

Sum-Squares for Error:

$$694 - (282 + 92 + 98 + 50 + 34) = 694 - 556 = 138$$

ANALYSIS OF VARIANCE

Source	Degrees of freedom	Sum of squares	Mean square	"F" Found	"F" Required	Remarks
Rows	(3+3) 6	92	...	....	....	....
Columns	(3+3) 6	98	...	....	....	....
Squares	1	50	...	....	....	....
Phosphate	1	50	50	4.3	4.75	Not significant
Potash	1	200	200	17.4	4.75	Significant
Interaction (Phosphate and Potash)	1	32	32	2.8	4.75	Not significant
Interaction (Treatment and Squares)	3	34	...	....	....	....
Error	12	138	11.5	....	....	....
Total	31	694				

Since the effect of potash is thus shown to be significant, a significant amount of difference between the potash totals would be $t\sqrt{\text{Error Mean Square} \times n \times 2}$ or $2.18\sqrt{11.5 \times 16 \times 2}$ or 41.8 tons.

Thus the difference (1260 — 1180) of 80 tons (an average for the 16 plots of 5 T.C.A.) is indicated to be a reliable gain that is most probably the result of the potash fertilization.

The response to phosphate is not significant, and there is no indication that a response from either one of these minerals is affected by the presence or absence of the other, i.e., there is no interaction between phosphate and potash.

Example No. 3

Example No. 3 is of a 2 × 3 Factorial Experiment in which any 2 Factors* ("A" and "B") are tested at all of any 3 Amounts ("1," "2," and "3"). Thus there are 6 combined Treatments, as follows:

A1	A2	A3
B1	B2	B3

* Such as nitrogen, or phosphate, or potash, or lime, or forms, or methods, or varieties, etc.

These 6 treatments are shown in a balanced arrangement in 5 Blocks with a total of 30 plots:

TREATMENT IDENTITY AND YIELDS AS T.S.A.

Block I		Block II		Block III		Block IV		Block V	
A1	7.1	B3	8.0	A1	7.3	B3	8.0	A1	6.0
B2	8.4	A2	7.4	B2	7.7	A2	7.4	B2	7.5
A3	8.7	B1	7.2	A3	9.6	B1	7.2	A3	8.4
B3	8.1	A1	6.9	B3	8.8	A1	7.0	B3	8.4
A2	8.0	B2	7.6	A2	7.7	B2	7.8	A2	7.2
B1	7.6	A3	8.4	B1	7.5	A3	9.2	B1	7.4

These data may be set up for Totals as follows:

Block	A1	A2	A3	B1	B2	B3	Block totals
I	7.1	8.0	8.7	7.6	8.4	8.1	47.9
II	6.9	7.4	8.4	7.2	7.6	8.0	45.5
III	7.3	7.7	9.6	7.5	7.7	8.8	48.6
IV	7.0	7.4	9.2	7.2	7.8	8.0	46.6
V	6.0	7.2	8.4	7.4	7.5	8.4	44.9
Treatment totals	34.3	37.7	44.3	36.9	39.0	41.3	233.5

Factor "A" Totals $= 34.3 + 37.7 + 44.3 = 116.3$
Factor "B" Totals $= 36.9 + 39.0 + 41.3 = 117.2$
Amount "1" Totals $= 34.3 + 36.9 = 71.2$
Amount "2" Totals $= 37.7 + 39.0 = 76.7$
Amount "3" Totals $= 44.3 + 41.3 = 85.6$

Work Sheet for Example No. 3:

Correction factor: $\dfrac{(233.5)^2}{30} = 1817.41$

Total Sum-Squares:

$(7.1)^2 + (6.9)^2 + (7.3)^2 + \ldots (8.0)^2 + (8.4)^2 = 1833.33$
-1817.41

Corrected 15.92 (with 29 degrees of freedom)

Sum-Squares Blocks:

$\dfrac{(47.9)^2 + (45.5)^2 + (48.6)^2 + (46.6)^2 + (44.9)^2}{6} = \dfrac{10914.19}{6} = 1819.03$
-1817.41

Corrected 1.62 (with 4 degrees of freedom)

Sum-Squares Treatments:

$\dfrac{(34.3)^2 + (37.7)^2 + (44.3)^2 + (36.9)^2 + (39.0)^2 + (41.3)^2}{5} =$

$\dfrac{9148.57}{5} = 1829.71$
-1817.41

Corrected 12.30 (with 5 degrees of freedom)

Sum-Squares Factors (A and B):

$$\frac{(116.3)^2 + (117.2)^2}{15} = \frac{27261.53}{15} = 1817.44$$

$$-1817.41$$

Corrected .03 (with 1 degree of freedom)

Sum-Squares Amounts (1, 2, and 3):

$$\frac{(71.2)^2 + (76.7)^2 + (85.6)^2}{10} = \frac{18279.69}{10} = 1827.97$$

$$-1817.41$$

Corrected 10.56 (with 2 degrees of freedom)

Sum-Squares Interaction (Factors and Amounts):

$12.30 - (.03 + 10.56) = 1.71$ [with $5 - (1 + 2)$ or 2 degrees of freedom]

Sum-Squares Error:

$15.92 - (1.62 + .03 + 10.56 + 1.71) = 2.00$
[with $29 - (4 + 1 + 2 + 2)$ or 20 degrees of freedom]

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Sum squares	Mean square or variance	"F" Found	"F" Required	Remarks
Blocks	4	1.62	....	....	....	....
"Factors"	1	.03	.03	.3	4.35	Not significant
"Amounts"	2	10.56	5.28	52.8	3.49	Significant
Interaction	2	1.71	.86	8.6	3.49	Significant
Error	20	2.00	.10	....	....	....
Total.	29	15.92				

This analysis indicates that the "Factors" themselves do not show a significant difference, but that the "Amounts" do have real different effects upon the yields. A difference between the "Amounts" totals greater than

$t\sqrt{\text{Error Mean Square} \times n \times 2}$ or $2.09\sqrt{.10 \times 10 \times 2}$ which is 2.95 tons would be significant. Hence it is clear that both "2" and "3" are better than "1," and also that "3" is better than "2."

Since this analysis also shows a definite interaction between the two factors and the amounts at which they were tested, we may look immediately for this interaction. A significant amount of difference between any two of the combined-treatment totals would be $t\sqrt{\text{Error Mean Square} \times n \times 2}$ or $2.09\sqrt{.10 \times 5 \times 2}$ which amounts to 2.09 tons. A comparison of the various combined-treatment totals shows which differences are significant. Thus, Factor "B" is superior to "A" by $(36.9 - 34.3)$ 2.6 or an average of $(2.6 \div 5)$ .52 T.S.A. when the Amount is "1," but when the Amount is "3" we note just the reverse situation, i.e., "A" is better than "B" by an average of $\left(\frac{44.3 - 41.3}{5}\right)$ .60 T.S.A. However, with the Amount at "2" the two factors do not differ significantly.

Example No. 4

For our fourth example, we use a 4×2 Factorial Experiment which would be suitable for testing 4 Methods (A, B, C, D) of using 2 Amounts of Nitrogen (1, 2).

The 8 possible treatment combinations, i.e., A1, B1, C1, D1, A2, B2, C2, D2, might be laid down in an arrangement in 4 Blocks with 32 plots as follows:

YIELDS AS T.S.A.

I	A1	5.1	D2	5.2	C2	6.0	B1	6.0
	B2	5.8	C1	6.4	D1	5.6	A2	4.8
II	C2	6.1	B1	5.7	A1	5.5	D2	5.4
	D1	5.1	A2	5.7	B2	5.9	C1	6.9
III	A1	4.9	D2	5.7	C2	5.6	B1	5.9
	B2	6.1	C1	6.0	D1	5.9	A2	5.3
IV	C2	5.2	B1	5.1	A1	4.6	D2	4.9
	D1	5.0	A2	4.8	B2	5.4	C1	5.9

IDENTITIES

A = applied in 1 dose, at 3½ mos.

B = applied in 2 doses, at 1½ and 3½ mos.

C = applied in 3 doses, at 1½, 3½, and 6 mos.

D = applied in 3 doses, at 1½, 3½, and 10 mos.

1 = 150 lb N

2 = 200 lb N

ARRANGEMENT OF YIELD DATA TO SECURE TOTALS

Block	A1	B1	C1	D1	A2	B2	C2	D2	Block totals
I	5.1	6.0	6.4	5.6	4.8	5.8	6.0	5.2	44.9
II	5.5	5.7	6.9	5.1	5.7	5.9	6.1	5.4	46.3
III	4.9	5.9	6.0	5.9	5.3	6.1	5.6	5.7	45.4
IV	4.6	5.1	5.9	5.0	4.8	5.4	5.2	4.9	40.9
Treatment totals ...	20.1	22.7	25.2	21.6	20.6	23.2	22.9	21.2	177.5

"Methods" totals:

"A" = 20.1 + 20.6 = 40.7

"B" = 22.7 + 23.2 = 45.9

"C" = 25.2 + 22.9 = 48.1

"D" = 21.6 + 21.2 = 42.8

"Nitrogen" totals:

"1" = 20.1 + 22.7 + 25.2 + 21.6 = 89.6

"2" = 20.6 + 23.2 + 22.9 + 21.2 = 87.9

Work Sheet for Example No. 4:

Correction factor: $\frac{(177.5)^2}{32} = 984.57$

Total Sum-Squares:

$(5.1)^2 + (5.5)^2 + (4.9)^2 + \ldots (5.7)^2 + (4.9)^2 =$ 993.09

-984.57

Corrected 8.52 (with 31 degrees of freedom)

Sum-Squares Blocks:

$\frac{(44.9)^2 + (46.3)^2 + (45.4)^2 + (40.9)^2}{8} = \frac{7893.67}{8} =$ 986.71

-984.57

Corrected 2.14 (with 3 degrees of freedom)

Sum-Squares Treatments:

$\frac{(20.1)^2 + (22.7)^2 + (25.2)^2 + \ldots (21.2)^2}{4} = \frac{3957.35}{4} =$ 989.34

-984.57

Corrected 4.77 (with 7 degrees of freedom)

Sum-Squares Methods:

$$\frac{(40.7)^2 + (45.9)^2 + (48.1)^2 + (42.8)^2}{8} = \frac{7908.75}{8} = 988.59$$

$$-984.57$$

Corrected 4.02 (with 3 degrees of freedom)

Sum-Squares Nitrogen:

$$\frac{(89.6)^2 + (87.9)^2}{16} = \frac{15754.57}{16} = 984.66$$

$$-984.57$$

Corrected .09 (with 1 degree of freedom)

Sum-Squares Interaction (Methods and Nitrogen):

$4.77 - (4.02 + .09) = .66$ [with $7 - (3 + 1)$ or 3 degrees of freedom]

Sum-Squares Error:

$8.52 - (2.14 + 4.02 + .09 + .66) = 1.61$ [with $31 - (3 + 3 + 1 + 3)$ or 21 degrees of freedom]

ANALYSIS OF VARIANCE

Source	Degrees of freedom	Sum squares	Mean square	"F" Found	"F" Required	Remarks
Blocks	3	2.14	....	....	....	....
Methods	3	4.02	1.34	16.8	3.07	Significant
Nitrogen	1	.09	.09	1.1	4.32	Not significant
Interaction (M × N)	3	.66	.22	2.8	3.07	Not significant
Error	21	1.61	.08	....	....	....
Total	31	8.52				

This analysis indicates a definite effect of Methods but no effect from the Amount of nitrogen nor any significant interaction between methods and amounts.

A significant amount of difference between the Methods totals would be $2.08\sqrt{.08 \times 8 \times 2}$ or 2.35 tons. Hence we have these comparisons:

"C" over "B" by (48.1 — 45.9) 2.2 tons — not significant
"C" over "D" by (48.1 — 42.8) 5.3 tons — significant (Avg. 0.66 T.S.A.)
"C" over "A" by (48.1 — 40.7) 7.4 tons — significant (Avg. 0.93 T.S.A.)
"B" over "D" by (45.9 — 42.8) 3.1 tons — significant (Avg. 0.39 T.S.A.)
"B" over "A" by (45.9 — 40.7) 5.2 tons — significant (Avg. 0.65 T.S.A.)
"D" over "A" by (42.8 — 40.7) 2.1 tons — not significant

This indicates that both "B" and "C" were significantly better methods for applying the nitrogen than either "A" or "D."

Example No. 5

Our fifth example is a 3 × 3 Factorial Experiment with 3 Varieties (A, B, X) and 3 Amounts of Nitrogen (1, 2, 3) in a special arrangement known as a Graeco-Latin Square. In this example we have used 36 plots in 4 Graeco-Latin Squares for the 9 possible Treatment combinations of these factors which are identified as:

A1	A2	A3
B1	B2	B3
X1	X2	X3

YIELDS AS T.S.A.

G. L. SQUARE I

A1	8.01	B2	8.60	X3	7.65
B3	8.26	X1	7.41	A2	8.67
X2	7.62	A3	8.83	B1	8.04

G. L. SQUARE II

B2	9.99	A1	8.22	X3	8.71
X1	8.00	B3	9.27	A2	9.61
A3	9.14	X2	8.61	B1	8.18

B2	9.64	X3	9.02	A1	8.13
X1	8.43	A2	9.06	B3	9.10
A3	8.72	B1	8.77	X2	8.60

G. L. S. III

X3	8.37	A1	8.10	B2	9.01
A2	8.76	B3	8.34	X1	7.64
B1	7.83	X2	8.48	A3	8.61

G. L. S. IV

These data may be set up as follows:

Treatments	Square I	Square II	Square III	Square IV	Treatment totals	Variety totals
A1	8.01	8.22	8.13	8.10	32.46	
A2	8.67	9.61	9.06	8.76	36.10	
A3	8.83	9.14	8.72	8.61	35.30	
Total A						103.86
B1	8.04	8.18	8.77	7.83	32.82	
B2	8.60	9.99	9.64	9.01	37.24	
B3	8.26	9.27	9.10	8.34	34.97	
Total B						105.03
X1	7.41	8.00	8.43	7.64	31.48	
X2	7.62	8.61	8.60	8.48	33.31	
X3	7.65	8.71	9.02	8.37	33.75	
Total X						98.54
Square totals	73.09	79.73	79.47	75.14		
Grand total						307.43

Nitrogen Totals

"1" = (32.46 + 32.82 + 31.48) = 96.76
"2" = (36.10 + 37.24 + 33.31) = 106.65
"3" = (35.30 + 34.97 + 33.75) = 104.02

Work Sheet for Example No. 5:

Correction factor: $\frac{(307.43)^2}{36} = 2625.37$

Total Sum-Squares:

$(8.01)^2 + (8.67)^2 + (8.83)^2 + (8.04)^2 + \ldots (8.48)^2 + (8.37)^2 =$
2637.84
—2625.37

Corrected 12.47 (with 35 degrees of freedom)

Sum-Squares for Graeco-Latin Squares:

$$\frac{(73.09)^2 + (79.73)^2 + (79.47)^2 + (75.14)^2}{9} = \frac{23660.52}{9} = 2628.95$$

$$-2625.37$$

Corrected 3.58 (with 3 degrees of freedom)

Sum-Squares Treatments:

$$\frac{(32.46)^2 + (36.10)^2 + (35.30)^2 + \ldots (33.75)^2}{4} = \frac{10529.43}{4} = 2632.36$$

$$-2625.37$$

Corrected 6.99 (with 8 degrees of freedom)

Sum-Squares Varieties only:

$$\frac{(103.86)^2 + (105.03)^2 + (98.54)^2}{12} = \frac{31528.33}{12} = 2627.36$$

$$-2625.37$$

Corrected 1.99 (with 2 degrees of freedom)

Sum-Squares Nitrogen only:

$$\frac{(96.76)^2 + (106.65)^2 + (104.02)^2}{12} = \frac{31556.88}{12} = 2629.74$$

$$-2625.37$$

Corrected 4.37 (with 2 degrees of freedom)

Sum-Squares for Interaction (Varieties and Nitrogen):

$6.99 - (1.99 + 4.37) = .63$ [with $8 - (2 + 2)$ or 4 degrees of freedom]

Sum-Squares for Error:

$12.47 - (3.58 + 1.99 + 4.37 + .63) = 1.90$ [with $35 - (3 + 2 + 2 + 4)$ or 24 degrees of freedom]

ANALYSIS OF VARIANCE

Source	Degrees of freedom	Sum of squares	Mean square	"F" Found	"F" Required	Remarks
Graeco-Latin Squares..	3	3.58	....	....	....	....
Varieties	2	1.99	1.00	12.5	3.40	Significant
Nitrogen	2	4.37	2.19	27.4	3.40	Significant
Interaction (V × N) ..	4	.63	.16	2.0	2.78	Not significant
Error	24	1.90	.08	....	....	....
Total.....	35	12.47				

There is no interaction between these 3 varieties and the amounts of nitrogen at which they were tested.

A significant amount of difference (t × SEd) for the Variety and Nitrogen totals would be $2.06\sqrt{.08 \times 12 \times 2}$ or 2.86 tons.

Hence we find both varieties "A" and "B" better than "X" but not dissimilar between themselves; also nitrogen levels "2" and "3" better than "1," but no real difference between "2" and "3."

This same arrangement is a proper one for the analysis of variance as made for multiple Latin squares. In such a case the detail would be set up with degrees of freedom as follows:

Source	Degrees of freedom	
Squares	3	
Rows (2 + 2 + 2 + 2)	8	
Columns (2 + 2 + 2 + 2)	8	
Treatments	8	Varieties 2 Nitrogen 2 Interaction (V × N) 4
Error	8	
Total	35	

Unless the mean variance for rows and columns is quite large, it may be best to leave same with the Error variance to insure a more conservative estimate of the treatment effects. Thus one can "go too far" in breaking down the Error variance, and so reduce this measure to a point beyond where it is practically no measure at all.)

Example No. 6

For our sixth example we have selected a plan for a 4 × 3 Factorial experiment which should be suitable for testing 4 varieties (A, B, C, D) with 3 Amounts of Nitrogen (1, 2, 3). This calls for 12 combined Treatments:

A1	A2	A3
B1	B2	B3
C1	C2	C3
D1	D2	D3

These 12 treatments may best be installed in a split-plot arrangement. We have used 4 blocks, with 16 whole-plots divided so as to furnish 48 split-plots.

In any split-plot arrangement, those factors between which the greater yield differences are to be expected should be tested in the whole-plots, while those for which the smaller differences are expected will be tested in the split-plots. This is desirable since the number of replications of the split-plots is greater than the replicates of whole-plots:

Block 1				Block 2				Block 3				Block 4			
A1	66	B3	80	C3	80	D1	61	C1	62	B3	80	A3	81	D1	69
A2	77	B2	74	C2	81	D2	82	C2	88	B2	82	A2	76	D2	72
A3	79	B1	71	C1	70	D3	81	C3	81	B1	79	A1	63	D3	74
D3	77	C1	72	B1	83	A3	85	D3	84	A1	73	B1	75	C3	73
D2	77	C2	81	B2	78	A2	83	D2	77	A2	84	B2	78	C2	74
D1	60	C3	74	B3	75	A1	70	D1	70	A3	83	B3	85	C1	64

DETAILS FOR THIS ANALYSIS OF VARIANCE

Source	Degrees of freedom	
Blocks	3	
Varieties	3	
Error (a)	9	(Total degrees of freedom for whole plots = 15)
Nitrogen	2	
Interaction (3 × 2) (Varieties and Nitrogen)	6	
Error (b)	24	
Total	47	

Note that:

(1) Degrees of freedom for Error (a) = Degrees of freedom for Blocks × Degrees of Freedom for Varieties, e.g., 3 × 3.

(2) Degrees of freedom for Error (b) = Degrees of freedom for Blocks × Sum of Degrees of Freedom for both Nitrogen and Interaction, e.g., 3 × (2 + 6).

SET-UP FOR SECURING TOTALS

Varieties	Block No.	Nitrogen Treatment No. 1	Nitrogen Treatment No. 2	Nitrogen Treatment No. 3	Whole plot totals	Variety totals
A	1	66	77	79	222	
	2	70	83	85	238	
	3	73	84	83	240	
	4	63	76	81	220	
Total		272	320	328		920
B	1	71	74	80	225	
	2	83	78	75	236	
	3	79	82	80	241	
	4	75	78	85	238	
Total		308	312	320		940
C	1	72	81	74	227	
	2	70	81	80	231	
	3	62	88	81	231	
	4	64	74	73	211	
Total		268	324	308		900
D	1	60	77	77	214	
	2	61	82	81	224	
	3	70	77	84	231	
	4	69	72	74	215	
Total		260	308	316		884
Nitrogen totals		1108	1264	1272	Grand total	3644

Blocks:	1	2	3	4	
	222	238	240	220	
	225	236	241	238	
	227	231	231	211	
	214	224	231	215	
Block totals	888	929	943	884	(3644)

Work Sheet for Example No. 6:

Correction factor: $\frac{(3644)^2}{48} = 276640$

Total Sum-Squares:

$(66)^2 + (70)^2 + (73)^2 + (63)^2 + (71)^2 - \ldots (84)^2 + (74)^2 = 278836$

-276640

Corrected 2196 (with 47 degrees of freedom)

Sum-Squares for Blocks:

$\frac{(888)^2 + (929)^2 + (943)^2 + (884)^2}{12} = 276858$

-276640

Corrected 218 (with 3 degrees of freedom)

Sum-Squares for Whole Plots:

$\frac{(222)^2 + (238)^2 + (240)^2 + \ldots (231)^2 + (215)^2}{3} = 277101$

-276640

Corrected 461 (with 15 degrees of freedom)

Sum-Squares for Varieties:

$\frac{(920)^2 + (940)^2 + (900)^2 + (884)^2}{12} = 276788$

-276640

Corrected 148 (with 3 degrees of freedom)

Sum-Squares for Error (a) (for Varieties):

$461 - (218 + 148) = 95$ [with $15 - (3 + 3)$ or 9 degrees of freedom]

Sum-Squares for Treatments:

$\frac{(272)^2 + (308)^2 + (268)^2 + \ldots (308)^2 + (316)^2}{4} = 278140$

-276640

Corrected 1500 (with 11 degrees of freedom)

Sum-Squares for Nitrogen:

$\frac{(1108)^2 + (1264)^2 + (1272)^2}{16} = 277709$

-276640

Corrected 1069 (with 2 degrees of freedom)

Sum-Squares for Interaction ($V \times N$):

$1500 - (148 + 1069) = 283$ [with $11 - (3 + 2)$ or 6 degrees of freedom]

Sum-Squares Error (b) *(for Nitrogen and Interaction):*

$2196 - (218 + 148 + 95 + 1069 + 283) = 383$

[with $47 - (3 + 3 + 9 + 2 + 6)$ or 24 degrees of freedom]

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Total sum squares	Mean square	"F" Found	"F" Required	Remarks
Blocks	3	218	....	....	....	....
Varieties	3	148	49.3	4.65	3.86	Significant
Error (a)	9	95	10.6	....	....	....
Nitrogen	2	1069	534.5	33.41	3.40	Significant
Interaction ($V \times N$)	6	283	47.2	2.95	2.51	Significant
Error (b)	24	383	16.0	....	....	....
Total	47	2196				

For a significant amount of difference between the 3 Variety totals we would need $t\sqrt{\text{Error (a) Mean Square} \times n \times 2}$ or $2.26\sqrt{10.6 \times 12 \times 2}$ which is 36 tons. Hence we note the following:

"B" is better than "C" or "D" but not better than "A."

"A" is also probably superior to "D" but not to "C."

There is no difference between "D" and "C."

A significant amount of difference between the 3 Nitrogen totals would be $t\sqrt{\text{Error (b) Mean Square} \times n \times 2}$ or $2.06\sqrt{16.0 \times 16 \times 2}$ which is 46.6 tons. Thus Nitrogen Nos. 2 and 3 are both better than No. 1, but Nos. 2 and 3 are not significantly different themselves.

The evidence of a significant interaction between the varieties and nitrogen suggests that their response is not always the same, and so we look further to see just what this difference is. A significant amount of difference between the 12 treatment totals would be $2.06\sqrt{16.0 \times 4 \times 2}$ or 23.3 tons. And so we note that Variety "B" is superior to "A," "C," and "D" with Nitrogen No. 1 but not with the other two amounts of nitrogen.

Example No. 7

For the seventh example, we apply the analysis of variance to the results which have been secured from the same group of plots which have received the same treatment for a series of years: in other words, to the analysis of a Serial Experiment. This procedure should be an exceedingly valuable one for the plantation agriculturist who has accumulated results of this nature and has been somewhat at a loss to interpret them collectively.

For this example we have used an experiment in which there were 3 Treatments (R, S, T) which had been harvested for 4 years (31, 33, 35, 37). The data were secured from an arrangement of 21 plots in 7 Blocks, as follows:

Block I			Block II			Block III			Block IV		
T	S	R	T	S	R	T	S	R	T	S	R
			R	S	T	R	S	T	R	S	T
			Block V			Block VI			Block VII		

The yields for each Treatment in each Block for each Year are arranged for totals as follows:

Year	Block	Treatment R	Treatment S	Treatment T	Block totals	
1931	1	85	86	93	264	
	2	92	86	89	267	
	3	86	94	89	269	
	4	91	88	76	255	
	5	89	86	94	269	
	6	83	91	87	261	
	7	80	88	79	247	
Treatment totals		606	619	607	1832	Year total

		Treatment	Treatment	Treatment	Block	
1933	1	85	84	101	270	
	2	86	99	93	278	
	3	85	94	104	283	
	4	88	85	99	272	
	5	83	97	91	271	
	6	83	89	98	270	
	7	82	99	84	265	
Treatment totals		592	647	670	1909	Year total
1935	1	111	94	111	316	
	2	89	107	102	298	
	3	99	90	112	301	
	4	82	89	108	279	
	5	87	94	97	278	
	6	95	97	106	298	
	7	78	112	88	278	
Treatment totals		641	683	724	2048	Year total
1937	1	90	79	101	270	
	2	82	92	82	256	
	3	104	87	88	279	
	4	80	78	86	244	
	5	75	91	88	254	
	6	73	83	99	255	
	7	74	96	75	245	
Treatment totals		578	606	619	1803	Year total
Treatment totals for four years		2417	2555	2620	7592	Grand total

Work Sheet for Example No. 7:

Correction factor: $\frac{(7592)^2}{84} = \frac{57638464}{84} = 686172$

Total Sum-Squares:

$(85)^2 + (92)^2 + (86)^2 + (91)^2 + \ldots (99)^2 + (75)^2 = 693142$

-686172

Corrected 6970 (83 degrees of freedom)

Sum-Squares Blocks and Years:

$\frac{(264)^2 + (267)^2 + (269)^2 + \ldots (245)^2}{3} = \frac{2066538}{3} = 688846$

-686172

Corrected 2674 (27 degrees of freedom)

Sum-Squares Combined Years × Treatments:

$\frac{(606)^2 + (592)^2 + (641)^2 + \ldots (619)^2}{7} = \frac{4822846}{7} = 688978$

-686172

Corrected 2806 (11 degrees of freedom)

Sum-Squares Treatments:

$\frac{(2417)^2 + (2555)^2 + (2620)^2}{28} = \frac{19234314}{28} = 686940$

-686172

Corrected 768 (2 degrees of freedom)

Sum-Squares Years:

$$\frac{(1832)^2 + (1909)^2 + (2048)^2 + (1803)^2}{21} - \frac{14445618}{21} = 687887$$

$$-686172$$

Corrected 1715 (3 degrees of freedom)

Sum-Squares Blocks:

(Sum-Squares Blocks and Years) — (Sum-Squares Years) = 2674 — 1715 = 959
(27 — 3 = 24 degrees of freedom)

Sum-Squares Interaction (Years × Treatment):

(Sum-Squares Combined Years × Treatments) — (Sum-Squares Years + Sum-Squares Treatments) = 2806 — (1715 + 768) = 2806 — 2483 = 323
[11 — (3 + 2) = 6 degrees of freedom]

Sum-Squares Error:

(Total Sum-Squares) — [Sum-Squares Blocks + Sum-Squares Treatments + Sum-Squares Years + Sum-Squares Interaction (Y + T)] = 6970 — (959 + 768 + 1715 + 323) = 6970 — 3765 = 3205
[83 — (24 + 2 + 3 + 6) = 48 degrees of freedom]

ANALYSIS OF VARIANCE

Source	Degrees of freedom	Sum squares	Mean square	"F" Found	"F" Required	Remarks
Blocks	24	959	....	....	....	....
Treatments	2	768	384	5.73	3.18	Significant
Years	3	1715	572	8.54	2.79	Significant
Interaction (treatments × years)	6	323	54	.81	2.29	No effect
Error	48	3205	67	....	....	....
Total	83	6970				

$$\text{SEd for } \textit{Treatment Totals} = \sqrt{\text{Error Mean Square} \times n \times 2}$$

$$= \sqrt{67 \times 28 \times 2} = \sqrt{3752} = 61.3$$

Amount needed for significance = t × SEd = 2.01 × 61.3 = 123.2 tons

Differences:

Treatment S over R = 2555 — 2417 = 138 tons (Avg. $\frac{138}{28}$ = 4.93 T.C.A.) Significant

Treatment T over S = 2620 — 2555 = 65 tons (Avg. $\frac{65}{28}$ = 2.32 T.C.A.) Not significant

$$\text{SEd for } \textit{Year Totals} = \sqrt{67 \times 21 \times 2} = \sqrt{2814} = 53.1$$

For significance: t × SEd = 2.01 × 53.1 = 106.7 tons

Also, but of lesser interest, is the fact that the 1935 yields were definitely higher than those for 1933, 1931, and 1937; but there was no real difference between the 1931, 1933 and 1937 yields.

There was no effect of interaction between treatments and years.

Example No. 8

For our eighth and final example, we offer a more complex Factorial Arrangement in which 3 Factors (N, P, K) are all combined at each of 2 Levels (1, 2);

this gives 8 combined Treatments which we have arranged on an area of 32 plots in 4 Blocks as indicated:

YIELDS AS T.C.A.

Block I

A	71	C	75
J	74	L	81
K	77	M	78
B	75	D	77

Block II

D	84	B	71
M	87	K	76
L	84	J	79
C	77	A	74

Block III

C	72	A	70
L	82	J	78
M	80	K	71
D	76	B	68

Block IV

B	70	D	64
K	73	M	84
J	72	L	79
A	66	C	71

Treatment identity	Combination
A	N1 + P1 + K1
B	N1 + P2 + K1
C	N1 + P1 + K2
D	N1 + P2 + K2
J	N2 + P1 + K1
K	N2 + P2 + K1
L	N2 + P1 + K2
M	N2 + P2 + K2

PLOT YIELDS AS SET UP FOR TOTALS

Block	A	B	C	D	J	K	L	M	Block totals
I	71	75	75	77	74	77	81	78	608
II	74	71	77	84	79	76	84	87	632
III	70	68	72	76	78	71	82	80	597
IV	66	70	71	64	72	73	79	84	579
Treatment totals	281	284	295	301	303	297	326	329	2416

The next step is to secure the proper data for use in calculating the treatment effects for the main factors as well as for the 2- and 3-Factor combinations. This is most easily done by setting up the following tabular arrangement:

COMBINATION OF TREATMENTS TO SHOW MAIN EFFECTS AND DOUBLE AND TRIPLE INTERACTIONS

Effect of	A	B	C	D	J	K	L	M
N	—	—	—	—	+	+	+	+
P	—	+	—	+	—	+	—	+
K	—	—	+	+	—	—	+	+
NP	+	—	+	—	—	+	—	+
NK	+	+	—	—	—	—	+	+
PK	+	—	—	+	+	—	—	+
NPK	—	+	+	—	+	—	—	+

This table must be correctly made up. The first step is to assign the proper + and — signs for each of the 3 main effects (N, P, and K): a — sign is placed in the proper columns under the Treatment Identity headings, for each Level "1,"

and a + sign for each Level "2" which is associated with each main effect factor indicated in the left-hand column.

The next step is to determine the + and — signs for the 2-factor effects (NP, NK, and PK). This is done (1) by assigning a + sign when the main effect signs of the two factors concerned are the same, i.e., when the main effect signs are both + or both —, and (2) by assigning a — sign when they are different.

Similarly, for the 3-factor effect (NPK), the sign will be + when its 2-factor and main effect both have the same sign, and — when they have different signs.

These signs are necessary in order to calculate the treatment effects shown in the next table. This table is made up by combining the treatment totals according to the + and — signs in the above plan for determining the main and interaction effects.

CALCULATION OF TREATMENT EFFECTS

For	A	B	C	D	J	K	L	M	Treatment effect*
N	—281	—284	—295	—301	+303	+297	+326	+329 =	94
P	—281	+284	—295	+301	—303	+297	—326	+329 =	6
K	—281	—284	+295	+301	—303	—297	+326	+329 =	86
NP	+281	—284	+295	—301	—303	+297	—326	+329 =	—12
NK	+281	+284	—295	—301	—303	—297	+326	+329 =	24
PK	+281	—284	—295	+301	+303	—297	—326	+329 =	12
NPK	—281	+284	+295	—301	+303	—297	—326	+329 =	6

* The total difference between the higher and the lower level.

Perhaps these interaction effects will be more easily understood if we examine them from the properly chosen levels of the Treatment totals as follows:

Treatment identity	Levels of N	Levels of P	Levels of K	Total yield
A	1	1	1	281
B	1	2	1	284
C	1	1	2	295
D	1	2	2	301
J	2	1	1	303
K	2	2	1	297
L	2	1	2	326
M	2	2	2	329

(1) The total NP interaction will be the difference between the sum of those treatments which have a similar level of N and P, and the sum of the treatments having different levels of N and P, e.g.

$$(A + C + K + M) - (B + D + J + L) = 1202 - 1214 = -12 \text{ tons}$$

Furthermore, with P1, we have a gain for N2 over N1 of 53 tons:

$$(J + L) - (A + C) = 629 - 576 = 53,$$

while with P2, we have a gain for N2 over N1 of only 41 tons:

$$(K + M) - (B + D) = 626 - 585 = 41.$$

Hence the difference of N2 over N1 was less by (53 — 41) 12 tons in the presence of high phosphate (P2) than with low phosphate (P1).

Conversely, with N1, we have a gain for P2 over P1 of 9 tons:

$$(B + D) - (A + C) = 585 - 576 = 9,$$

while with N2, we have a loss for P2 over P1 of —3 tons:

$$(K + M) - (J + L) = 626 - 629 = -3.$$

Thus in the presence of high nitrogen (N2) there were 12 tons less cane from the high-phosphate (P2) than from the low-phosphate (P1) treatment.

(2) The total NK interaction will be the difference between the sum of the treatments having the same levels of N and K, and the sum of the treatments having different levels of N and K, e.g.

$$(A + B + L + M) - (C + D + J + K) = 1220 - 1196 = 24.$$

With K1, we have a gain of 35 tons for N2 over N1, e.g.

$$(J + K) - (A + B) = 600 - 565 = 35.$$

With K2, the gain for N2 over N1 was 59 tons, e.g.

$$(L + M) - (C + D) = 655 - 596 = 59.$$

Hence the difference of N2 over N1 was greater by (59 — 35) 24 tons in the presence of high potash (K2) than with low potash (K1).

Similarly it may be shown that the difference between K2 and K1 was greater by 24 tons in the presence of high nitrogen (N2) than with low nitrogen (N1), e.g.

$$\begin{aligned}(C + D) - (A + B) &= 596 - 565 = 31\\ (L + M) - (J + K) &= 655 - 600 = 55\\ 55 - 31 &= 24.\end{aligned}$$

(3) The total PK interaction amounting to 12 tons may be shown as follows:

$$(A + J + D + M) - (B + K + C + L) = 1214 - 1202 = 12.$$

With P1, there is a gain of 37 tons for K2 over K1, while with P2 this gain for K2 over K1 is 49 tons; hence a difference of 12 tons favors K2 over K1 with the higher phosphate. Similarly, the difference of — 3 tons for P2 over P1 with K1, as compared with a corresponding gain of 9 tons with K2, indicates a 12-ton difference favoring P2 over P1 in the presence of the higher potash level.

(4) The 3-factor interaction of N, P, and K can be shown to be the difference between (a) 2 levels of one factor when the level of the other two factors is similar, and (b) the same 2 levels of the same factor when the level of the other two factors is different, e.g.

(a) The difference between N2 and N1, when each is associated with the same level of both P and K, was 50 tons:

$$(M + J) - (A + D) = 632 - 582 = 50.$$

(b) The difference between N2 and N1, when associated with a different level of both P and K, was 44 tons:

$$(K + L) - (C + B) = 623 - 579 = 44.$$

The total NPK interaction is therefore the difference between these two amounts, 50 — 44 or 6 tons.

Work Sheet for Example No. 8:

Correction factor: $\frac{(2416)^2}{32} = \frac{5837056}{32} = 182408$

Total Sum-Squares (with 31 degrees of freedom):

$(71)^2 + (74)^2 + (70)^2 + (66)^2 + (75)^2 + \ldots (80)^2 + (84)^2 =$ 183350
—182408

Corrected 942

Sum-Squares Blocks (with 3 degrees of freedom):

$\frac{(608)^2 + (632)^2 + (597)^2 + (579)^2}{8} = \frac{1460738}{8} =$ 182592
—182408

Corrected 184

Sum-Squares Treatments (with 7 degrees of freedom):

$\frac{(281)^2 + (284)^2 + (295)^2 + \ldots (329)^2}{4} = \frac{731778}{4} =$ 182945
—182408

Corrected 537

Sum-Squares Error (with 21 degrees of freedom):

$942 - (184 + 537) = 221$

PARTITION OF SUM SQUARES TREATMENTS*

		Sum squares
N	$(94)^2 \div 32 =$	276.12
P	$(6)^2 \div 32 =$	1.13
K	$(86)^2 \div 32 =$	231.13
NP	$(12)^2 \div 32 =$	4.50
NK	$(24)^2 \div 32 =$	18.00
PK	$(12)^2 \div 32 =$	4.50
NPK	$(6)^2 \div 32 =$	1.13
		536.51

Note: The sum of these Sum Squares (537) must check with the Sum-Squares Treatments as previously calculated.

The Analysis of Variance may then be set up as follows:

Source	Degrees of freedom	Sum squares	Mean square	"F" Found	"F" Required	Remarks
Blocks	3	184	....	....	....	....
N only	1	276	276	26.3	4.32	Significant
P only	1	1	1	.1	4.32	Not significant
K only	1	231	231	22.0	4.32	Significant
N and P	1	5	5	.5	4.32	Not significant
N and K	1	18	18	1.7	4.32	Not significant
P and K	1	5	5	.5	4.32	Not significant
N, P, and K	1	1	1	.1	4.32	Not significant
Error	21	221	10.5	....	....	....
Total	31	942				

* (Treatment effect)$^2 \div$ total number of plots.

Thus we note a reliable effect of nitrogen and of potash, but no significant interaction between them, nor is there any real influence shown by the phosphate applications.

The SEd for the main effect totals would be $\sqrt{10.5 \times 16 \times 2}$ or 18.3 tons and a significant amount of difference between same would be 2.08 $\times$ 18.3 or 38.1 tons. This makes both the difference between the two nitrogen levels (94) and that between the two potash levels (86) significant amounts; an average gain for the second levels over the first levels of $\left(\frac{94}{16}\right)$ 5.9 T.C.A. for nitrogen, and of $\left(\frac{86}{16}\right)$ 5.4 T.C.A. for potash.

Neither the 2-Factor effects nor the 3-Factor effect are significant. The total gain of 24 tons for the interaction of N and K is not a reliable one. (*If* any 2-Factor effect had been significant, its SEd would have been $\sqrt{10.5 \times 8 \times 2}$ or 13 tons and a significant amount of difference between the 2-Factor effect totals would have been 2.08 $\times$ 13 or 27 tons. And for a significant 3-Factor effect, a significant difference would have needed to be $2.08\sqrt{10.5 \times 4 \times 2}$ or 19.1 tons.)

Discussion

"Student's" method for analyzing the results of field experiments has been very popular with our plantation agriculturists. That it has a common basis with Fisher's analysis of variance can be shown by applying it to the same set of data we have used on page 79, and determining the standard error of the mean difference between the paired plot yields. This error will be found to be the same as the standard error of the difference determined from the mean Error Variance of the Analysis of Variance calculation, by the formula

$$\text{SEd} = \sqrt{\frac{\text{Error Variance} \times 2}{n}}$$

For example (Student's Method):

			Difference		
	"A"	"X"	"A" over "X"	d	d^2
	76	74	+ 2	.62	.3844
	81	79	+ 2	.62	.3844
	73	73	0	—1.38	1.9044
	76	71	+ 5	3.62	13.1044
	72	70	+ 2	.62	.3844
	77	83	— 6	—7.38	54.4644
	82	80	+ 2	.62	.3844
	74	70	+ 4	2.62	6.8644
Totals	611	600	+11		77.8752
Average	76.4	75.0			
Mean difference			+ 1.38		

From these data we may calculate a standard error (SEm) for this mean difference (1.37) as follows:

$$SEm = \sqrt{\frac{\text{Sum of } d^2}{n\,(n-1)}} = \sqrt{\frac{77.8752}{8 \times 7}} = \sqrt{1.39} = 1.18$$

Similarly, the standard error of a difference (SEd) between the two means, from the analysis of variance calculation, would be found from the formula:

$$SEd = \sqrt{\frac{\text{Error Variance} \times 2}{n}} = \sqrt{\frac{5.57 \times 2}{8}} = \sqrt{1.39} = 1.18$$

Thus we note that both methods give the identical standard error for the difference between treatments. This fact holds good, however, only when 2 treatments are being compared. When more than 2 treatments are being tested in the same experiment, this similarity does not hold because the analysis of variance uses the generalized error as secured from the pooled sum of squares in place of the individual error from pairs of plots as obtained in Student's method.

There is still some difference of opinion with regard to the preference for the generalized error (as obtained from the analysis of variance) and the individual error (as secured from Student's method). The variance method assumes that a more or less homogeneous error is associated with all treatments, and we know that this assumption may not always be true for differential treatments included in field tests with sugar cane. For instance, we have some indication from a study of more than 600 fertilizer experiments, that a higher standard error is more often associated with the lesser than with the greater amounts of fertilizer which were compared in these tests. We also recognize the fact that in variety testing the error for different canes may vary considerably; hence, one variety with a high error may give to an experiment such a large generalized error that the superiority of other varieties (with lower errors) will be unidentified. This apparent failure of the analysis of variance to identify a significant relationship, when the standard errors of the treatment yields are not homogeneous, may be illustrated with the following example of 3 Treatments (A, B, X) which were tested in 6 Blocks:

LAYOUT WITH PLOT YIELDS (T.C.A.)

I		II		III	
A	74	X	84	B	76
B	77	B	80	A	72
X	68	A	77	X	80
A	71	X	60	B	74
B	72	B	77	A	72
X	74	A	72	X	74
IV		V		VI	

SET-UP FOR TOTALS

Block	A	B	X	Block totals
I	74	77	68	219
II	77	80	84	241
III	72	76	80	228
IV	71	72	74	217
V	72	77	60	209
VI	72	74	74	220
Treatment totals	438	456	440	1334

Averages	(T.C.A.)
"A"	$73 \mp .9$
"B"	76 ∓ 1.1
"C"	73 ∓ 3.5

ANALYSIS OF VARIANCE

Source	Degrees of freedom	Sum squares	Mean square	"F" Found	"F" Required	Remarks
Blocks	5	201	....	....	....	....
Treatments	2	33	16.5	.73	4.10	Not significant
Error	10	226	22.6	....	....	....
Total	17	460				

This analysis indicates no effect of treatment.

Yet when these same data are examined by Student's method, we find that "B" is definitely superior to "A," viz.:

"A"	"B"	Difference "B" over "A"	d	d^2
74	77	+ 3	0	0
77	80	+ 3	0	0
72	76	+ 4	1	1
71	72	+ 1	2	4
72	77	+ 5	2	4
72	74	+ 2	1	1
Totals 438	456	+18		10
Average 73	76	+ 3		

$$SD = \sqrt{\frac{10}{6}} = 1.29$$

$$\text{"Z"} = \frac{3.0}{1.29} = 2.32$$

$$n = 6$$

Odds = 560 to 1, and therefore highly significant.

Similarly, using Fisher's "t" value to indicate significance we would have

$$SEd = \sqrt{\frac{10}{30}} = .58 \qquad t = \frac{\text{Difference}}{SEd} = \frac{3.0}{.58} = 5.2$$

and "t" required for n-1 or 5 degrees of freedom is only 2.57. Thus the difference found (3 T.C.A.) in favor of "B" is a highly significant one.

However, it is well to point out that unless there is a sound and logical reason for the high error of a Treatment average, low errors in the same test may also be due to chance and hence be unreliable. That is why the generalized error from the analysis of variance may be a safer figure to use than separately selected individual-treatment errors; and if perchance it appears that there are real differences within a test, there is no good reason why they should remain hidden, for it is still possible to make use of other statistical measures to determine the reliability of differences between the average yields.

Summary

In the preceding pages we have attempted to show by actual example how the results from field experiments can be set up and examined for evidence that the measured yield differences between treatments are really an effect of the treatments and not more likely due to chance. The use of a statistical measure as simply one more tool in the hands of the field investigator is presented in just that sense, and little effort is made to expound the basic theories of statistical analyses which

has been so aptly done by such modern leaders as "Student," Fisher, Yates, Love, Snedecor, Goulden, and others in their various publications.*

Of especial interest because it has made possible the intelligent use of factorial experiments, the "analysis of variance" method as developed by Fisher has been quite adequately exemplified. Its utilization for identifying the contributory causes of the total "error" in an experiment should result in our placing still more emphasis on the experimental plan before the field test is actually installed.

Finally, plans and analyses for the simpler forms of factorial experiments have been offered as a suggested improvement in our experimental technique which heretofore has confined itself to the testing of single issues in separate field tests. The greater precision which is possible in securing yield effects and in estimating their significance, should make these factorial experiments doubly attractive to the plantation agriculturist.

Addenda:

For reference, and as examples of acceptable arrangements for "Grade A" experiments, we offer the following designs for the more simple Factorial Experiments:

No. 1—For a 2 × 2 Factorial, e.g. {2 Varieties (A, X) at 2 Amounts of Nitrogen; 4 Treatments — 7 Blocks — 28 Plots.

A1	X1	A2	X2
A2	X2	A1	X1
X2	A2	X1	A1
X1	A1	X2	A2
A1	X1	A2	
A2	X2	A1	
X2	A2	X1	
X1	A1	X2	

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom	"F" required
Blocks	6	....
Varieties	1	4.41
Nitrogen	1	4.41
Interaction (V × N)	1	4.41
Error	18	....
Total	27	

* References which were freely consulted during the preparation of this discussion, and with which the student of statistical methods for field experiments should be familiar, are as follows:

1. Fisher, R. A., "The Design of Experiments" published by Oliver & Boyd, London, England.
2. Goulden, C. H., "Methods of Statistical Analysis" published by John Wiley and Sons, Inc., New York.
3. Love, H. H., "Application of Statistical Methods" published by Commercial Press, Shanghai, China.
4. Snedecor, G. W., "Statistical Methods" published by the Collegiate Press, Ames, Iowa.
5. Yates, F., "The Design and Analysis of Factorial Experiments" published by The Imperial Bureau of Soil Science, Harpenden, England.

No. 2—For a 2 × 3 Factorial, e.g. {2 Varieties (A, X) at 3 Amounts of Nitrogen; 6 Treatments — 5 Blocks — 30 Plots.

A1	X1	A3	X3	A1
X2	A2	X2	A2	X2
A3	X3	A1	X1	A3
X3	A3	X1	A1	X3
A2	X2	A2	X2	A2
X1	A1	X3	A3	X1

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom	"F" required
Blocks	4	....
Varieties	1	4.35
Nitrogen	2	3.49
Interaction (V × N).	2	3.49
Error	20	....
Total	29	

No. 3—For a 3 × 2 Factorial, e.g. {3 Varieties (A, X, B) at 2 Amounts of Nitrogen; 6 Treatments — 5 Blocks — 30 Plots.

A1	B2	A1	B2	A1
X2	X1	X2	X1	X2
B2	A1	B2	A1	B2
A1	B2	A1	B2	A1
X1	X2	X1	X2	X1
B2	A1	B2	A1	B2

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom	"F" required
Blocks	4	....
Varieties	2	3.49
Nitrogen	1	4.35
Interaction (V × N).	2	3.49
Error	20	....
Total	29	

No. 4—For a 4 × 2 Factorial, e.g. {4 Varieties (A, B, C, X) at 2 Amounts of Nitrogen; 8 Treatments — 4 Blocks — 32 Plots.

A1	X2	B2	C1
B2	C1	A1	X2
C2	B1	X1	A2
X1	A2	C2	B1
A1	X2	B2	C1
B2	C1	A1	X2
C2	B1	X1	A2
X1	A2	C2	B1

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom	"F" required
Blocks	3	....
Varieties	3	3.07
Nitrogen	1	4.32
Interaction (V × N).	3	3.07
Error	21	....
Total	31	

No. 5—For a 3 × 3 Factorial, e.g. {3 Varieties (A, X, B) at 3 Amounts of Nitrogen; 9 Treatments — 4 Graeco-Latin Squares — 36 Plots.

X1	B2	A3	
A2	X3	B1	I
B3	A1	X2	
B2	A3	X1	
A1	X2	B3	II
X3	B1	A2	
X3	A1	B2	
B1	X2	A3	III
A2	B3	X1	
A1	B2	X3	
B3	X1	A2	IV
X2	A3	B1	

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom	"F" required
G. L. Squares.....	3	....
Varieties	2	3.40
Nitrogen	2	3.40
Interaction (V × N).	4	2.78
Error	24	....
Total	35	

No. 6—For a 3-Factor, Complex experiment: {N, P, K, each at 2 Levels (1, 2); 8 Treatments—4 Blocks—32 Plots.

A	C	B	D	C	A
J	L	K	M	L	J
K	M	J	L	M	K
B	D	A	C	D	B
D	B				
M	K				
L	J				
C	A				

PREFERRED ANALYSIS OF VARIANCE

Source	Degrees of freedom
Blocks	3
Treatments*	7
Error	21
Total	31

Identity		Treatment
A	=	N1 P1 K1
B	=	N1 P2 K1
C	=	N1 P1 K2
D	=	N1 P2 K2

Identity		Treatment
J	=	N2 P1 K1
K	=	N2 P2 K1
L	=	N2 P1 K2
M	=	N2 P2 K2

* This will be partitioned to furnish 1 degree of freedom each for the following:

N P K
NP NK PK
NPK

A PARTIAL TABLE OF VALUES OF "F" AND "t" CORRESPONDING TO THE 5 PER CENT LEVEL OF SIGNIFICANCE

(Abstracted from Snedecor's* computations from Fisher's tables)

n2 \ n1	Values of "F" 1	2	3	4	5	6	Value of t
3	10.13	9.55	9.28	9.12	9.01	8.94	3.18
4	7.71	6.94	6.59	6.39	6.26	6.16	2.78
5	6.61	5.79	5.41	5.19	5.05	4.95	2.57
6	5.99	5.14	4.76	4.53	4.39	4.28	2.45
7	5.59	4.74	4.35	4.12	3.97	3.87	2.37
8	5.32	4.46	4.07	3.84	3.69	3.58	2.31
9	5.12	4.26	3.86	3.63	3.48	3.37	2.26
10	4.96	4.10	3.71	3.48	3.33	3.22	2.23
11	4.84	3.98	3.59	3.36	3.20	3.09	2.20
12	4.75	3.88	3.49	3.26	3.11	3.00	2.18
13	4.67	3.80	3.41	3.18	3.02	2.92	2.16
14	4.60	3.74	3.34	3.11	2.96	2.85	2.15
15	4.54	3.68	3.29	3.06	2.90	2.79	2.13
16	4.49	3.63	3.24	3.01	2.85	2.74	2.12
17	4.45	3.59	3.20	2.96	2.81	2.70	2.11
18	4.41	3.55	3.16	2.93	2.77	2.66	2.10
19	4.38	3.52	3.13	2.90	2.74	2.63	2.09
20	4.35	3.49	3.10	2.87	2.71	2.60	2.09
21	4.32	3.47	3.07	2.84	2.68	2.57	2.08
22	4.30	3.44	3.05	2.82	2.66	2.55	2.07
23	4.28	3.42	3.03	2.80	2.64	2.53	2.07
24	4.26	3.40	3.01	2.78	2.62	2.51	2.06
25	4.24	3.38	2.99	2.76	2.60	2.49	2.06
26	4.22	3.37	2.98	2.74	2.59	2.47	2.06
27	4.21	3.35	2.96	2.73	2.57	2.46	2.05
28	4.20	3.34	2.95	2.71	2.56	2.44	2.05
29	4.18	3.33	2.93	2.70	2.54	2.43	2.05
30	4.17	3.32	2.92	2.69	2.53	2.42	2.04
40	4.08	3.23	2.84	2.61	2.45	2.34	2.02
50	4.03	3.18	2.79	2.56	2.40	2.29	2.01

Footnote:

n1 = degrees of freedom associated with "Treatment" mean square
n2 = degrees of freedom associated with "Error" mean square
} when determining "F" required.

n2 = degrees of freedom for Error mean square where using "t".

* "Statistical Methods" by G. W. Snedecor (by permission).

Pythium Root Rot of Sugar Cane in Louisiana

(A Review by C. W. Carpenter)

A comprehensive account of *Pythium* root rot disease of sugar cane, as it concerns the Louisiana sugar cane industry, embodying the results of exhaustive studies and much thorough work since 1924, by Rands and Dopp,* was published in October 1938. The virtual bankruptcy of the Louisiana sugar industry in the period 1923 to 1926 is attributed by the authors to mosaic disease, red rot and root rot. The industry recovered with the introduction of mosaic-tolerant POJ varieties; however, some of these varieties later proved susceptible to red rot and root rot. The susceptible canes were replaced by the Coimbatore canes, Co. 281 and Co. 290, and varieties bred at Canal Point, ". . . under the coordinated sugar cane breeding and testing program of the United States Department of Agriculture, in cooperation with the Louisiana Agricultural Experiment Station and the American Sugar Cane League." The susceptibility of the POJ varieties and some of the recently propagated hybrids to root rot indicated the need for more knowledge of root rot, and of the nature of resistance to this disease, in order to maintain or improve yields.

Previous reports by Edgerton and his co-workers, and Rands and his associates, have shown that the most important root disease of sugar cane in Louisiana is caused by *Pythium arrhenomanes*. This species is now considered identical with the *Pythium* species causing root rot in Hawaii, reported in 1920, first under the name of *P. butleri,* later revised to *P. aphanidermatum* and *P. graminicolum,* in conformance with the progress of critical studies of closely related species by various investigators. It was not considered advisable to import cultures of parasitic cane fungi to make comparative studies in Hawaii. In some of the taxonomic studies conducted by Rands and his associates and in experiments, a culture of the cane *Pythium* from Hawaii was included for comparisons of morphological characters and pathogenicity. *P. arrhenomanes* has been definitely identified thus far in Hawaii, the Philippine Islands, Mauritius, Canada and the United States, in the latter two countries as a cause of root rot disease of maize and cereals.

The fungus *P. arrhenomanes* (more recently referred to in our reports as *P. graminicolum*) is widely distributed in the cultivated soils of Hawaii, if indeed it is not ubiquitous throughout local cane lands. In the current program of testing new varieties and hybrids of local propagation, *Pythium arrhenomanes* promptly eliminates the more susceptible varieties from further consideration. It is safe to predict that no variety commercially susceptible to *Pythium* root rot will be recommended for spreading on account of its performance in our variety and regional testing stations. *Pythium* root rot apparently will always remain a hazard to the indiscriminate spreading of canes of unknown resistance.

Rands and Dopp advance the tentative hypothesis that the strains of *P. arrhenomanes* recently isolated in Louisiana may be more virulent than those isolated in

* Rands, R. D. (Senior Pathologist) and Dopp, Ernest (Assistant Pathologist). Pythium Root Rot of Sugarcane. Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Technical Bulletin 666, 1938.

1932 and 1933, indicating possible adaptation to the newer varieties of cane. They state that evidence of physiologic specialization of the *Pythium sp.* and the influence on yields show root rot to be a dynamic rather than a static factor in sugar cane production. According to Rands and Dopp (p. 49): ". . . the greatest danger from physiologic specialization of *Pythium arrhenomanes* would appear to be in connection with the less resistant varieties, which thus far unfortunately have predominated among the many new seedling selections otherwise most promising for commercial use."

The importance of *Pythium* root rot to cane production in Hawaii when varieties are naturally susceptible or become so as a result of a modified soil environment, and the potential hazard of the disease to future crops, should it be a fact that more virulent strains of the parasite are developing by a process of adaptation to our resistant crop canes, are sufficient reasons to quote in full the summary of this valuable contribution to the study of the root rot problem.

Since the failure in 1923 to 1926 of the old noble varieties of sugarcane in Louisiana, which was due to combined damage from mosaic, red rot, and the so-called root disease, the last-mentioned trouble has continued to be a serious problem; this despite the restoration of the industry from the introduction of somewhat more resistant and vigorous hybrid canes. On the latter, root rot is obviously the most important factor in the root-disease complex, even in exceptional cases when the symptoms approach those characteristic of the condition on the old varieties.

An apparent increase in the severity of root rot on certain of the newer varieties following their widespread and continued cultivation has emphasized the need for fundamental knowledge about the disease as a necessary basis for determining the resistance of new seedling selections and securing and maintaining further yield improvement.

Root rot was noted to be causing widespread damage in Louisiana as early as 1908, when red rot of the seed cane was also reported. Combined damage from the two diseases during the period 1910-20 reduced State-wide average yields by 23 percent, and the subsequent mosaic epidemic another 30 percent, which brought the industry to virtual bankruptcy. Varietal introductions by the Department and cooperating agencies (Louisiana Agricultural Experiment Station and the American Sugar Cane League) have gradually restored production to approximately that of the earlier long-period level of 1888-1907.

On certain of the presently grown and moderately susceptible varieties, such as Co. 281 and C. P. 28/19, root rot is usually manifested merely by unthrifty appearance, deficient and delayed tillering (suckering), and closing in of the rows. During occasional bad root rot years yellowing of the leaves, severe wilting, and death of young plants may result in poor stands and virtual crop failure on heavy clay soils, due to complete destruction of roots on both seed cuttings and young shoots.

As indicated in preliminary reports, *Pythium arrhenomanes* Drechsler was found to be the principal cause of the root rot. Although during the past quarter century root-disease epidemics have been reported from most sugarcane-producing countries, this particular fungus has been identified only from Hawaii, the Philippine Islands, Mauritius, Canada (where it attacks cereals), and the United States.

Twelve additional species of *Pythium* and several other fungi were isolated from decaying roots obtained in surveys of the sugar- and sirup-producing sections of the Gulf States. They were most numerous in Louisiana where the roots had either been injured by the gnawing of minute soil fauna or were weakened by red rot of the cuttings or some unfavorable soil condition. Infection tests conducted under a wide range of environmental conditions in the greenhouse were negative to the extent of development of a general root rot characteristic of *P. arrhenomanes*. They also showed no tendency to act as secondary invaders to the latter fungus. However, under the predisposing influence of dilute concentrations of a soil toxin (salicylic aldehyde) severe root rot and appreciable reduction of plant weight were caused by several of these miscellaneous species, particularly *P. dissotocum* and *P. graminicolum*. These results in

conjunction with the survey records suggest that only under very abnormal conditions may any of these species contribute to an important extent in the destruction of sugarcane roots.

Physiologic specialization and, to some extent, varietal adaptation of *Pythium arrhenomanes* in the Louisiana sugar district have been revealed by greenhouse inoculation experiments with more than 200 isolates of this species obtained in root rot surveys of representative plantations. Significant differences in average virulence of the isolates were found to occur between different plantations or localities, as well as between an earlier (1927-31) and a more recent (1935-36) survey.

Since the latter finding could not readily be explained on the basis of attenuation, resulting from prolonged maintenance in artificial culture of the early collection, actual increase in average virulence of the fungus during the period of 5 to 7 years separating the surveys is tentatively assumed to have occurred. This is conceivably due in part at least to segregation and multiplication of certain biotypes brought about by general adoption of more resistant varieties, which permitted survival of only the more virulent or adaptable components of the earlier population of the fungus.

A serious decline in yield of the susceptible P. O. J. 234 in relation to the highly resistant Co. 290 and C. P. 807 varieties in replicated agronomic yield comparisons during the past 8 years has been associated with apparent increase in root rot severity, and possibly reflects in part at least the above-found increased virulence of the *Pythium*.

No increase in root rot of resistant varieties has been observed, although one isolate of the *Pythium* was found capable of seriously damaging the Co. 290 in greenhouse tests. However, this may represent merely a chance variant rather than a specialized subpopulation of more virulent forms in the fields.

Physiologic specialization of *Pythium arrhenomanes* and its potential influence on yields show that root rot must be looked upon as a dynamic rather than a static factor, as hitherto considered in relation to sugarcane production. Therefore root rot-resistance tests of new seedling selections necessitate prior artificial infestation of the soil with a collection of the most virulent locally known cultures of the fungus.

The apparent degree of resistance or susceptibility in field tests of well-known varieties, representing the recognized species of sugarcane, is given. Most noble varieties (*Saccharum officinarum*) were found to be highly susceptible, while the Chinese canes (*S. sinense*) and the wild sugarcane (*S. spontaneum*) were highly resistant. Two Indian varieties of *S. barberi* occupied an intermediate position.

F_1 hybrids from crosses between the susceptible, noble, and resistant wild cane were usually resistant, but successive backcrossing to the noble parent (''nobilization'') to secure commercial qualities gave increasing susceptibility in the few seedlings studied.

Since most elite breeding canes possess extremely complex inheritance, an important object of the Department's coordinated sugarcane breeding and disease-testing program is to discover more suitable parental combinations that will increase the chances of securing superior resistant varieties without increasing to prohibitive proportions the total number of seedlings to be tested.

Tentative root rot ratings on 111 first-year or later agronomic selections revealed nearly one-half to be resistant. If one or more of these should be found to combine the indispensable other qualities, especially early maturity, hazards from use of the presently available root rot-resistant varieties may be greatly minimized.

Among the present commercial varieties in Louisiana, Co. 290, C. P. 807, C. P. 28/11, and C. P. 29/116 are classed as resistant to root rot and also possess sufficient vigor for planting on the mixed and heavy soils. However, plantings of C. P. 807 have already been greatly diminished because of too great susceptibility to red rot. C. P. 29/320 has not been seriously damaged by root rot, but has been reported to be susceptible to red rot. Co. 281 and C. P. 28/19 are susceptible to root rot and ordinarily succeed only in light, well-drained soils.

Detailed studies confirmed the conclusions of other investigators that high winter rainfall and low spring temperatures greatly accentuate the damage to fall-planted cane. Summer planting of C. P. 28/19, when it must be grown on heavy soils, was found to prevent the serious losses in yield and the practical crop failure sometimes experienced with regular October plantings.

In controlled soil temperature tanks root rot was worst at 65° to 68° F., and became progressively less serious with increase in temperature to 97° which is past optimum for cane

growth. The effect of a more virulent strain of the *Pythium* was characterized by greater damage, particularly at intermediate and high temperatures, while the use of a more resistant variety tended to suppress the disease, particularly at these temperatures.

Greater severity of root rot on mixed and heavy clay root-rot soils emphasize the need for better drainage, deep preparation by tractors, and other measures to prevent practical water-logging during periods of prolonged rainfall. The greatly accentuating effect on root rot of toxic materials possibly accumulating under such deficient aeration has been indicated by greenhouse experiments.

Increased root rot of plant cane has been noted to result apparently from excessive nitrogen fertilization of the crop furnishing the seed.

The comparative unimportance of root rot on muck and peat soils is apparently ascribable (among other things) to their greater biological activity and possible antibiotic effect on spread of the *Pythium*.

Improvement of the physical, chemical, and biological conditions of the root rot soils by continued plowing under of all cane trash and by moderate applications of factory filter-press cake or stable manure, when also accompanied by good drainage, has markedly reduced root-rot damage of susceptible varieties.

Attempts to discover a soil treatment or other direct methods for control of root rot that would not be prohibitive in cost have been unsuccessful.

Influence of Potash Fertilization Upon the Production and Composition of Dry Matter

By R. J. Borden

Investigators continue to seek ways and means to make intelligent use of plant composition figures to guide their recommendations of specific fertilizers to meet apparent plant food deficiencies in cropped soils. Only a fair amount of success has been obtained because of the many involved relationships with plant composition. One of the factors which may be concerned is apparent in the results secured from a study recently completed, which shows the effects of potash fertilization upon the production of dry matter and its potash composition and total uptake, when the crop is harvested at different stages of maturity.

Soils:

Eight soils which were used in this study were obtained from Ewa Plantation Company. They all have an adequate supply of available phosphate and none are what we would consider to be greatly deficient in available potash. Briefly they are described by the following summary:

Field No.	Origin	Color	Texture	Available K20 (p.p.m.)	pH
B1	Residual	Dark red	Silty loam	250	7.2
B2	"	"	"	280	7.3
B3	"	"	"	120	7.2
B4	"	"	"	370	7.2
18A	Alluvial	Dark reddish-brown	Silty clay loam	120	7.2
18B	Marine sedimentary	Yellowish-red brown	"	110	8.2
25D	"	"	"	170	7.4
29	"	"	"	280	8.2

Procedure:

After thorough preparation, standard Mitscherlich pots were filled with these soils, and three series with two potash differentials for each were provided in triplicates, i.e., 9 pots were adequately fertilized, each with 1.5 grams of K20 from sulphate of potash, and 9 pots were given no potash fertilizer. All pots were planted with Sudan grass, 40 plants being allowed to develop in each pot. Nitrogen and phosphate fertilization, and all conditions during the ensuing growth periods were similar.

Series I was harvested 50 days after planting, at a time when the crop was still growing and definitely immature. Series II was harvested at 70 days, when the plants were considered to be fully mature, while series III was allowed to become overmature and was not taken off until 90 days. Dry weights were secured from each pot at harvest, both the weight of roots and the weight of leaves and stems (with seed) being secured. Samples of all dry material were analyzed by the Chemistry department for total K20 content. Thus it is possible to calculate the

amounts of potash taken up in the dry matter produced and to determine the significant relationships.

The Average Dry Weights:

The effect of potash on the production of dry matter of stems and leaves and also of roots may be summarized for all 8 soils as follows:

	Grams dry weight harvested	
Series I—(50 days):	With potash	Without potash
Stems and leaves	905.4	890.0
Roots	187.2	188.1
Total dry weight	1,092.6	1,078.1
Series II—(70 days):		
Stems and leaves	1,299.1	1,195.7
Roots	256.4	238.8
Total dry weight	1,555.5	1,434.5
Series III—(90 days):		
Stems and leaves	1,311.8	1,284.0
Roots	266.4	273.1
Total dry weight	1,578.2	1,557.1
Dry weight total—all series	4,226.3	4,069.7

A small but highly significant difference (average 6.5 grams, with odds of 500 to 1) is found in the dry-weight totals which is apparently the effect of the potash fertilization. This effect is chiefly due to the increase in the aboveground part of the crop rather than to the roots. Nevertheless, a definite positive relationship ($r = .78 \pm .04$) can be shown to exist between the weights of stems and leaves and the root weights from the 48 pots used in this study.

When the three series are studied separately we find that the differences between the total dry weights which favor the crops receiving potash, both in the immature series I and in the overmature series III, are not significant; the average differences of 1.8 grams and 2.6 grams respectively might easily occur by chance once in four times. On the other hand, the average difference of 15.1 grams in series II carries odds of better than 1000 to 1. This indicates that there may be some relationship between the effect of potash fertilization on the production of dry matter and the stage of the crop's development at the time it is harvested. If this indication can be reliably substantiated, it will need serious consideration.

A still further "break down" of the harvest data indicates that the differences in the average dry weights between the two treatments within each series also vary with the individual soils that were used. This is seen in the accompanying table. The reason for the negative signs in series III is not clear; a very small amount of seed shattering was not believed to be large enough to seriously affect these weights.

DIFFERENCES IN TOTAL DRY WEIGHTS (K20 OVER NO K20)
(FROM AVERAGES OF 3 POTS OF EACH TREATMENT)

Soil	Series I Grams	Series II Grams	Series III Grams
B1	— .6	+23.5[s]	+ 5.1
B2	— 1.3	+ 4.0	+14.1[s]
B3	+ 4.3	+21.6[s]	+ 6.5
B4	+ 2.5	+14.8[s]	—12.0
18A	+11.7[s]	+17.3[s]	+ 4.3
18B	+ 6.1	+26.0[s]	+13.4[s]
25D	— 2.1	+10.0[s]	— 4.1
29	— 6.1	+ 3.8	— 6.2

([s] = These differences are significant, i.e., greater than 2 x SEd.)

With but a few exceptions, none of which are significantly less, however, both treatments on all 8 soils show increased dry weights in both their aboveground portions and their roots, with their increased age at harvest. These increases are large and definitely significant for all 70-day harvests over their respective 50-day crop, but further gains for the 90-day harvests are smaller, and in those cases where potash was supplied are not significantly different.

AVERAGE GAIN IN TOTAL DRY WEIGHT—GRAMS

Treatment	For 70-over 50-day harvest	For 90-over 70-day harvest
With potash	57.8	2.6 (not significant)
Without potash	44.5	15.3

Potash in Dry Matter:

Both the percentage of potash in the dry matter and the total amount taken up by the crop were definitely influenced by the potash applications.

The percentage of K20 was substantially greater in the immature crops than in either the mature or overmature plants. In the dry matter of stems and leaves, the per cent K20 decreased directly with age of harvest. In the roots, the percentage of potash dropped significantly in all 16 comparisons of series II with series I, but it then increased significantly in all comparisons of series III with series II. This apparent return of potash to the roots after 70 days growth is most interesting. Differences in the percentage of K20 between the plants of the two treatments were consistently less as the harvests were delayed.

A summary of the per cent K20 found in the dry matter, averaged for all 8 soils appears as follows:

	Per cent of K20		Difference
	With potash	Without potash	(K20 over no K20) Per cent
Series I—(50 days):			
Stems and leaves	2.008	.994	+1.014
Roots	.845	.422	+ .423
Total dry weight (true average)	1.763	.857	+ .906
Series II—(70 days):			
Stems and leaves	1.346	.723	+ .623
Roots	.375	.226	+ .149
Total dry weight (true average)	1.184	.644	+ .540
Series III—(90 days):			
Stems and leaves	1.104	.545	+ .559
Roots	.494	.374	+ .120
Total dry weight (true average)	.991	.514	+ .477

In these total dry weights, we find a significant decrease to an extent of 32 and 25 per cent respectively for the potash and no potash treatments in the percentage potash composition of the plants harvested at 70 days over the immature 50-day series, and a further 16 and 20 per cent decrease respectively for plants with and without potash fertilization, when the harvest is delayed from 70 to 90 days. This change in the percentage composition of potash which occurs with the stage of maturity is an important factor that will need to be considered when comparisons are being made from percentage data in plant composition studies.

The total potash recovered in the dry matter was quite definitely influenced by the potash applications. The amounts found in the total dry weight are not significantly different for the immature and mature plants but they are definitely less in the overmature crop. In the stems and leaves, there is less potash in the overmature (90-day) plants but the roots of these plants contain a larger amount than their respective 70-day series.

The total amount of potash recovered from the dry matter harvested from all 8 soils indicates some potash losses in the plant material which became greater as the harvest was delayed. This may be seen from the following summary. The apparent build-up of potash in the roots of the overmatured plants (series III) is also shown:

	Grams of K20 in dry matter		Grams K20 recovered	Grams K20 not recovered from amounts added
	With potash	Without potash		
Series I—(50 days):				
Stems and leaves.....	17.717	8.464		
Roots	1.545	.770		
Total dry weight....	19.262	9.234	10.028	1.972
Series II—(70 days):				
Stems and leaves.....	17.468	8.670		
Roots	.948	.563		
Total dry weight....	18.416	9.233	9.183	2.817
Series III—(90 days):				
Stems and leaves.....	14.347	6.991		
Roots	1.294	1.008		
Total dry weight....	15.641	7.999	7.642	4.358

Since soil analyses after harvest indicated that less than .13 gram of available K20 remained in any pot, regardless of the amount which had originally been supplied, or the length of the growing period, it appears that some of this fertilizer which was supplied is unaccounted for, in terms of available potash. For the 50-day series, this loss amounts to approximately only 17 per cent, but for the 70- and 90-day harvests, losses of 23 and 37 per cent respectively are noted. Since our pot technique precludes the loss of any leachates we can only speculate as to where this potash has gone.

Correlation:

There was no relationship between the final dry weights and the amount of potash contained therein. For the 24 pots which received no potash the correlation

coefficient (r) was only .05 ± .13; and for the soils which were fertilized with potash, r was .02 ± .14. Apparently this is an indication of the so-called "luxury consumption" of the available potash supply.

Summary:

1. Small gains that were secured from potash fertilization in the production of dry matter from eight soils, which are not markedly deficient in available potash, were significant only when the crop was harvested at its optimum period of development, i.e., neither immature nor overmature.

2. Although potash fertilization increased both the percentage and the total amount of this mineral found in the dry matter at harvest, there was no correlation between potash composition and final dry weights.

3. The percentage of potash in the total dry matter decreased quite substantially as the age of harvest was delayed. This was directly true for the leaves and stems, but in the roots there was apparently a return of potash when the plants passed their full maturity.

4. The total amount of potash, that was recovered in the total dry matter harvested, decreased with the age at harvest, i.e., the amount not recovered from that which had been supplied became increasingly greater as the crop reached and then passed maturity.

5. Such effects of potash fertilization as we have discussed are quite apt to introduce a complication into any attempt to interpret potash composition figures.

The Growth of Plants in Water and Sand Cultures

By J. P. Martin and C. W. Carpenter

The growth of plants in water and sand cultures was started 50 years ago or more, although in recent years it has received a great deal of study and enthusiastic publicity. In the literature this technique is referred to as soilless or tray agriculture, bath tub or tank farming, chemiculture, liquid or sand culture, and recently as hydroponics (hydro = water; ponics = made with).

Many plants are now grown experimentally in water and sand cultures and a number of commercial ventures with such methods are being made on the Mainland. The method has its limitations and in order for it to be successful various procedures have to be carefully followed. Anyone trying to grow plants for the first time by the water- or sand-culture method will experience disappointments which can only be avoided by experimenting and selecting the technique best suited for his conditions. The whole system is in many ways quite flexible and the most satisfactory results are to be obtained by trial and error. If one undertakes such a project without having had previous experience, it is suggested that plants at first be grown on a small scale so that a knowledge of the plant's requirements will be secured. Experience, after all, is the best teacher.

Plant Requirements

Plant growth is governed by light, temperature, moisture, and the physical and chemical qualities of the soil, all of which go to make up the environment; a change in any one of these factors may influence the rate of growth. In soilless agriculture the light and temperature factors must be favorable for plants. The chemical composition of the nutrient solution in which the plants are to be grown is comparable to the chemical qualities of the soil solution and is of paramount importance at all times to the development of the plant.

The chemical elements essential for plant growth are: hydrogen, oxygen, carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and iron; in addition to these are the trace elements: manganese, boron, zinc, copper and perhaps others. Hydrogen is obtained chiefly from water, oxygen from water and air, while carbon is taken in by the leaves from the air as carbon dioxide. The other elements are taken from the soil. For normal growth in water cultures or in the soil these elements must be present in sufficient quantities and in available forms. A deficiency or an excess of any one element produces an abnormal growth and frequently these abnormalities prove extremely useful in determining the plant's requirements or in diagnosing physiological diseases such as nitrogen or iron deficiency, as described below.

The Soluble Plant Fertilizer

In recent years, sugar cane has been successfully grown in water and sand cultures with various nutrient solutions at the Experiment Station, Hawaiian Sugar Planters' Association. Other plants such as tomatoes, potatoes, and asparagus

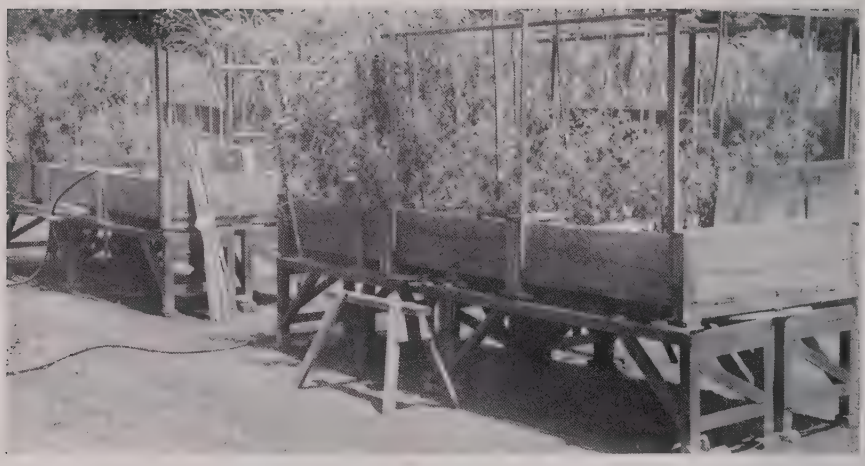

Fig. 1. Young tomato plants growing out of doors in black sand and irrigated with the Soluble Plant Fertilizer solution.

Fig. 2. Young tomato plants growing within a screened house in black sand and irrigated with a nutrient solution prepared from the S. P. Fertilizer.

which were used in special studies developed normally in sand cultures when irrigated with the nutrient solutions used for sugar cane.

With the thought in mind that a complete, soluble plant fertilizer might be useful for growing various plants without soil, as well as for stimulating the growth of potted plants, a water-soluble fertilizer was prepared, after a number of trials, from commercial chemicals. The various elements in this mixture are present in amounts equal to those in the nutrient solution which proved to be best adapted for sugar

carries both known and undetermined trace elements, has proved superior for plant growth in a number of cases to nutrient solutions prepared with chemically pure salts.

The S.P. Fertilizer is packed in air-tight containers, labeled, respectively, A, which contains the nitrogen, and B, which contains the other elements essential for growth; it may be obtained with directions for its use from the Pacific Guano and Fertilizer Company. The mixture has been prepared so that one teaspoonful each of A and B to two gallons of water gives a concentration satisfactory for plant growth. For some plants it might be advisable to make the nutrient solution slightly more acid; this is accomplished by adding dilute sulphuric acid to the tap water prior to the addition of the fertilizer; for example: if the original pH value of the tap water is 8.0, then 5 c.c. of normal sulphuric acid is usually required to bring the final pH value of the nutrient solution to 5.3 (further directions are given below).

Plants grown in sand cultures are irrigated lightly two or three times a week with the S.P. Fertilizer solution. As the plants begin to grow they are irrigated twice a week and supplied with tap water if they require additional moisture (Figs. 1 and 2).

If plants are grown in water cultures the containers are nearly filled with the solution which is renewed every ten days or two weeks. The frequency at which the solutions are changed will depend largely on the size of the plants, their rate of growth and the volume of solution in the containers. If deep containers are used some means for aerating the solutions should be provided. Details relating to aerating solutions, the size of containers, and mechanical supports for the plants are discussed below.

Tomatoes have been grown mainly in sand cultures and the yields have been quite satisfactory. Various stages of growth of the plants and the type of containers employed are shown in Figs. 1 and 2.

For potted plants the solution containing the S.P. Fertilizer is applied directly to the soil; an application once every two weeks has been found to be satisfactory for a number of the ordinary house plants but more or less frequent applications may be made according to the growth response desired.

The commercial fertilizers for soilless agriculture now on the market contain most of the elements listed above under "Plant Requirements," but the concentration of each element in one mixture may vary considerably from that in another mixture without greatly affecting the growth of the plant—in other words there is, as a rule, a margin of safety in the concentrations of each element for plant growth. A modification of the concentrations of one or more elements may be necessary to meet the requirements of some plants.

Water vs. Sand Cultures

Plants absorb the mineral nutrients from the soil solution through their roots. One important function of the soil is to accommodate a large root surface for absorption; another is to serve as a physical support or anchorage for the plant. The soil is to a greater or lesser degree self-aerating and self-draining. Plants grow well in water culture and in sand culture but some means of physical support must be provided in water culture. In the sand-culture method the quartz, beach, or black (volcanic cinders) sand provides physical support; the culture solution, added fre-

cane growth. This mixture, known as the *Soluble Plant (S.P.) Fertilizer,* which quently or continuously by mechanical means, provides the nutrients. If coral sand is to be used as a medium it should first be thoroughly washed with tap water and then rinsed with acidified water before attempting to grow plants in it. Coral sand selected from a locality well removed from the ocean where it has been exposed to rains is preferable to sand taken from the ocean's edge.

The most common design for water cultures includes a water-tight tank of metal or wood with a movable screened frame which fits the top. On the screened frame is placed a layer of excelsior, shavings, or other similar inert material (the substratum) in which the seed is planted or the seedlings transplanted; the roots penetrate this layer and are constantly submerged in the culture solution. For small plants various containers such as crocks or fruit jars may be used.

The tanks may be of various materials and of various shapes and sizes according to the size and number of plants to be grown. The tank should be water tight, the material non-corrosive, or covered with a neutral and safe coating such as a pure non-oily asphalt paint. Paint with a lead or other metallic base should be avoided. Galvanized iron and redwood, unless coated as described above, are unsatisfactory. The depth of the solution required varies somewhat with the root habits of the plants to be grown. Tubers and bulbs develop in the excelsior or other substratum and only the roots penetrate into the solution; for such crops a proportionately deeper layer of substratum is needed. Relatively shallow tanks provide sufficient depth without requiring excessive quantities of nutrient solution in proportion to the surface available for plant growth.

The tray may be made to cover the tank, or it may be made slightly smaller, to slip inside and rest on supports just above the solution. Sufficient space should be provided at the end of the tray to permit inspection and changing the solution. Aeration of the solution is necessary especially if deep containers are used. In small tanks the tray may be lifted several times a day so that the roots may carry air into the solution, or in larger tank cultures mechanical stirring, circulation of the solution by pumps, or bubbling air through the solution may be used to provide adequate circulation and aeration. Mosquito larvae (wigglers) frequently develop in water cultures unless the plants are grown in a screened house or the access of mosquitoes to the culture solution is otherwise prevented. The larvae cling to the roots and are not easily eliminated when the solutions are renewed.

Sand cultures are in many ways preferable to water cultures; the plants are supported by the sand, and the aeration as well as drainage are taken care of during the irrigation. The solution may be allowed to drip slowly onto the sand, collected below the cultures and used repeatedly—the drip method. With the aid of circulating pumps the solution may be applied continuously by drip or faster irrigation. Lack of aeration frequently accompanied by stagnation of the solution and decomposition of the roots is avoided to a large extent by using sand cultures.

Pests and Diseases: Plants grown in soilless culture are not immune to the diseases and insect pests of the field. Since plants are concentrated in a limited area, the pests may spread easily but they are more readily observed and measures for control may be more easily applied. Plants grown under cover, e. g., in a glass house, are particularly subject to infestation with plant lice and mites. Frequent sprayings of the foliage with water aid to simulate rain and dew conditions of the

field and tend to restrict the insect infestations. Spraying with nicotine-sulfate solution and dusting with sulphur may be required occasionally to control aphis and mites. The plants may be sprayed with Bordeaux mixture if fungous diseases are troublesome.

When sand is used for three months or more the root knot nematode may attack the roots of the plants and prevent normal growth or even kill the plants. Sand infested with nematodes, after continuous growth of a susceptible plant for several months, must be sterilized by heat or chemical disinfectants before satisfactory growth of any susceptible plant can be expected. Many vegetables are particularly susceptible to nematode attack, for example: tomatoes, potatoes, etc.

Expense: The cost of small outfits for either water or sand cultures in the home is nominal. Commercial units are expensive and still in the experimental stage. Before the venture is attempted on a commercial scale, a site should be selected near a good market for out-of-season products which command and receive premium prices.

Practical Uses of Soilless Agriculture

At the present time it may be said that soilless agriculture is an interesting hobby which produces edible or other useful products. Such culture is distinctly advantageous where neither good soil nor water are available in quantity (certain Pacific Islands). In favorable localities near large cities some commercial "tank farms" are being operated successfully by experienced growers of fancy agricultural products.

Any one who becomes interested in soilless agriculture should not be influenced by the too-enthusiastic propaganda written by laymen who eulogize tank farms and optimistically predict the ultimate substitution of tank culture for field agriculture. A statement has been made by a Mainland authority that it would require 20,000 times the annual production of phosphate in the United States to grow all of our vegetables by the tank-culture method.

For an entertaining and popular discussion of the water-culture method of growing plants the reader is referred to an article by Frank J. Taylor entitled "You Can Try It Yourself" in the *Saturday Evening Post,* August 20, 1938.

Preparation of Culture Solutions (Hoagland and Arnon)

A brief account of the water-culture method of growing plants, by D. R. Hoagland and D. I. Arnon of the College of Agriculture of the University of California, appeared in the C.R.E.A. (Committee on the Relation of Electricity to Agriculture) News Letter for June 1938. The authors state that many of the popular articles on the water-culture method of crop production are "grossly inaccurate in fact, and misleading in implications."

We are indebted to Hoagland and Arnon for the following formulae and directions for their use; the first formula we quote for the benefit of those who may wish to prepare culture solutions from fertilizers and chemicals of ordinary grade; the second, a more technical formula, we quote for use in schools where laboratory facilities are available.

Preparation of Nutrient Solutions: Method A, for Amateurs

Either one of the solutions given in Table V may be tried. The "T.C." solution may often be preferred because the ammonium salt delays the development of undesirable alkalinity. The salts are added to the water, preferably in the order given.

To either of the solutions add the elements iron, boron, manganese, zinc, and copper, which are required by plants in minute quantities. There is danger of toxic effects if much greater quantities of these elements are added than indicated later in the text.

With the exception of iron, the elements of this group are added only when the solution is first prepared or when the whole solution is changed.

TABLE V. COMPOSITION OF NUTRIENT SOLUTIONS
(The amounts given are for 25 gallons of solution)

Salt	Grade of salt	Approx. amt. in ounces	Approx. amount in tablespoons
	"P.N." Solution		
Potassium phosphate (monobasic)	Technical	½	1 level
Potassium nitrate	Fertilizer	2	4 level (of powd. salt)
Calcium nitrate	Fertilizer	3	7 level
Magnesium sulfate (Epsom salt)	Technical	1½	4 level
	"T.C." Solution		
Ammonium phosphate (monobasic)	Technical	½	1 heaping
Potassium nitrate	Fertilizer	2½	5 level (of powd. salt)
Calcium nitrate	Fertilizer	2½	6 level
Magnesium sulfate (Epsom salt)	Technical	1½	4 level

It may be necessary to add the iron solution at frequent intervals; for example, once or twice a week. If the leaves of the plant tend to become yellow the reason may be lack of iron, although a yellowing or mottling of leaves can also be due to other causes.

1. Iron Solution.

Dissolve a level teaspoon of iron tartrate (iron citrate or iron sulfate can be substituted, but the tartrate or citrate are often more effective than the sulfate) in a quart of water. Add half a cupful of this solution to 25 gallons of nutrient solution each time iron is needed (once weekly, or more frequently if the plants are pale).

2. Boron Solution.

Dissolve a level teaspoon of powdered boric acid in a gallon of water. Use a pint and a half of this solution for each 25 gallons of nutrient solution.

3. Manganese Solution.

Dissolve a teaspoon of crystalline, chemically pure manganese chloride ($MnCl_2 \cdot 4\,H_2O$) in a gallon of water. Manganese sulfate can also be used. Dilute one part of this solution with two parts of water, by volume. Use a pint of the *diluted* solution for each 25 gallons of water.

4. Zinc Solution.

Dissolve a level teaspoon of crystalline, chemically pure zinc sulfate ($ZnSO_4 \cdot 7\,H_2O$) in a gallon of water. Use four teaspoons of this solution for each 25 gallons of nutrient solution.

5. Copper Solution.

Dissolve a teaspoon of chemically pure copper sulfate ($CuSO_4 \cdot 5\,H_2O$) in a gallon of water. Dilute one part of this solution with four parts of water; use one *teaspoon* of the *diluted* solution for each 25 gallons of nutrient solution.

Testing and Adjusting the Acidity of Water and Nutrient Solution

The chemicals required are:

1. Brom thymol blue indicator. This can be obtained, with directions for use, from chemical supply houses, in the form of solutions or impregnated strips of paper.

2. Sulfuric acid. Purchase a supply of three per cent (by volume) acid of chemically pure grade. (Concentrated, chemically pure sulfuric acid may be purchased and diluted to three per cent strength, *but the concentrated acid is dangerous to handle by inexperienced persons*). This three per cent acid may be further diluted with water if a preliminary test indicates that only small additions of acid are required to bring about a desirable reaction.

Adjust the acidity of the water before adding nutrient salt according to directions given.

Test the degree of acidity of a measured sample of the water (a quart, for example) by noting the color of the added indicator or test paper immersed in the solution.

A yellow color indicates the desired slight acidity (with no further adjustment necessary), green a neutral reaction, blue an alkaline reaction.

Add the dilute sulfuric acid (three per cent or less) slowly with stirring until the original green or blue color *just* changes to yellow. Do not add more acid beyond this point, since the yellow color will also persist when excessive amounts of acid are added. Record the amount of acid required.

Finally add a proportionate amount of the acid to the solution in the culture tank or vessel, having first determined how much it holds.

The reaction of the culture solution should be likewise tested from time to time and, if found alkaline, corrected by the addition with stirring of dilute sulfuric acid. If strips of indicator paper are used, the test may be performed directly in the tank, or on a small sample of the culture solution.

Preparation of Nutrient Solutions: Method B, for Special Experimentation by Schools, etc.

The use of distilled water and chemically pure salts is recommended. Molal stock solutions (except when otherwise indicated) are prepared for each salt, and the amounts indicated below are used.

"P.N." solution	Cc. per liter of nutrient solution
M/1 KH_2PO_4 potassium acid phosphate	1
M/1 KNO_3 potassium nitrate	5
M/1 $Ca(NO_3)_2$ calcium nitrate	5
M/1 $MgSO_4$ magnesium sulfate	2
"T.C." solution	
M/1 $NH_4H_2PO_4$ ammonium acid phosphate	1
M/1 KNO_3 potassium nitrate	6
M/1 $Ca(NO_3)_2$ calcium nitrate	4
M/1 $MgSO_4$ magnesium sulfate	2

To either of these solutions add the following:

(*a*) Iron in the form of 0.5 per cent iron tartrate solution or other suitable iron salt, at the rate of 1 cc. per liter, about once weekly or as indicated by appearance of plants (more if pale).

(*b*) Prepare a supplementary solution which will supply boron, manganese, zinc, and copper, as follows:

Compound	Grams dissolved in 1 liter of H_2O
H_3BO_3	2.86
$MnCl_2 \cdot 4\,H_2O$	1.81
$ZnSO_4 \cdot 7\,H_2O$	0.22
$CuSO_4 \cdot 5\,H_2O$	0.08

Use 1 cc. of this solution for each liter of nutrient solution. This will give the following concentrations:

Element	Parts per million of nutrient solution
Boron	0.5
Manganese	0.5
Zinc	0.05
Copper	0.02

Adjustment of Reaction During Growth of Plants

If the culture solution should become alkaline (pH greater than 7) as a result of growing plants, make the solution slightly acid (about pH 6) by adding N/10 H_2SO_4 (or some other suitable dilution).

Changes of Nutrient Solution

As the plants begin to grow, nutrient salts will be absorbed and the acidity of the solution will change. More salts and acid may be added, but to know how much, chemical tests on the solution are required. When these cannot be made, an arbitrary procedure may be adopted of draining out the old solution every week or two, immediately refilling the tank with water, and adding salts and acid, as at the beginning of the culture. The number of changes of solution required will depend on size of plants, how fast they are growing, and on volume of solution. Distribute the salts and acid to different parts of the tank. In order to effect proper mixing, it may be well to fill the tank at first only partly full (but keep most of the roots immersed) and then after adding the salts and acid, to complete the filling to the proper level with a rapid stream of water.

In December 1938, Circular 347, entitled "The Water Culture-Method for Growing Plants Without Soil" by D. R. Hoagland and D. I. Arnon was published by the University of California. The authors have presented the principles and application of the water-culture method together with its possibilities and limitations, and have given detailed directions for growing plants by this method. This article should be extremely valuable to those interested in soilless agriculture.

Variation in Available Nutrients in an Uncropped Surface Soil

By R. J. Borden

It seems desirable to know the possible extent of the natural variation in the available soil nutrient content of a soil, so that a more reliable interpretation can be given to results of soil analyses when they are being used to guide fertilizer practices.

In a previous study (1), we have reported upon the seasonal variations in the availability of the principal nutrients in a soil while a cane crop was being grown thereon. In this present study (2) we have attempted to secure a similar picture from a soil which was maintained in bare fallow.

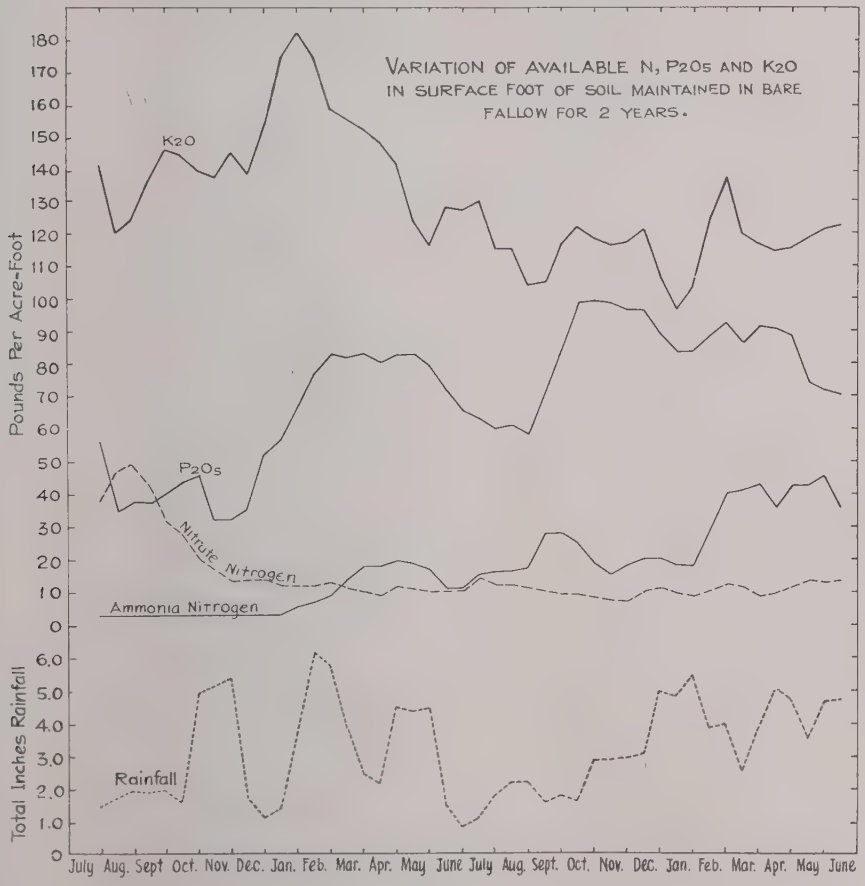

Fig. 1.

(1) Reported in The Hawaiian Planters' Record, 41: 47–55, 1937.

(2) Experiment Station, H.S.P.A., Project A–105—No. 82.

We selected a small area approximately 25 feet square at our Kailua substation, removed all plant growth, and maintained a clean surface soil thereon for a period of 2 years. During this period, twice each month, two separate soil sample composites from 10 auger borings each were taken from each of three soil depths: (a) 0-6″, (b) 6″-12″; and (c) 12″-24″. Auger holes from which soil was taken were refilled and marked with wire pins, so that each subsequent boring could be made not more than 6 or 8 inches from the previous bore. With such a procedure, the successively taken soil samples are believed to be quite comparable.

Soil samples thus taken (3) were analyzed (4) by rapid chemical methods in our soils laboratory. Results in detail are given in Table I, as pounds of available nutrients per acre in the three soil depths indicated.

A somewhat clearer picture of the variations that were found may be obtained from Fig. 1, which shows a smoothed curve (from moving averages) for the amounts of various nutrients found in the upper foot of soil, i.e., the total found in the 0-6″ and the 6″-12″ depths. As a matter of interest, a smoothed curve for rainfall is also included.

It is quite apparent that a variation in the available nutrient supply of this soil existed during the two-year period while it was being studied. The tabulated data indicate the range of this variability to be as follows:

For potash: from 75 lbs. on January 18, 1938 to 200 lbs. on January 18, 1937.
For phosphate: from 33 lbs. on August 18, 1936 to 107 lbs. on April 2, 1938.
For ammonia nitrogen: from less than 5 lbs. prior to February 1937 to 53 lbs. on May 5, 1938.
For nitrate nitrogen: from 6 lbs. on April 2, 1938 to 56 lbs. on August 3, 1936.

The differences in soil pH were perhaps not significant, but rainfall per half-month period varied from a half-inch to twelve inches.

Although from a strictly quantitative standpoint these ranges may appear large, the facts are not quite so disconcerting if we recognize the limitations in the practical application of soil analytical data and look at them from a qualitative or perhaps a semi-quantitative point of view. For instance: our qualitative grouping* of soils with respect to the availability of nutrients as found by R.C.M. analyses would indicate that the potash supply during the first year's samplings varied principally *within* the "doubtful" group, reaching 200 pounds only once and never going below 100 pounds. During the second year's sampling, the potash content of the periodic sample was quite generally *within* the "low" group, and it never got above 150 pounds. Thus from a practical standpoint the analyses during both years consistently call our attention to the fact that the available potash supply was inade-

(3) Taken by various assistant-agriculturists-in-training under supervision of K. H. Berg or L. R. Smith, and Y. Yamasaki.

(4) All R.C.M. analyses by H. M. Lee.

* Pounds per Acre-Foot of Available Nutrients by R.C.M.

Soil group	P_2O_5	K_2O	N
Low	0–20	0–125	0–25
Doubtful	21–35	126–200	26–50
Medium	36–100	201–300	51–100
High	100+	300+	100+

quate, from which we know that potash fertilization will be necessary for successful cane growth on this soil.

In the same way, the analyses of the periodic samples show that the phosphate supply varied considerably, but that quite generally all of the differences were well *within* the "medium" phosphate grouping. Only two samples actually got into the "high" group, and these showed amounts that are only just over the border of the "medium" group upper limit, i.e., 104 and 107 pounds respectively; similarly only three samples were below the lower limit of the "medium" group, i.e., at 33, 34, and 35 pounds respectively. Hence the soil in this area is shown to have a fair amount of available phosphate but not sufficient to warrant omitting phosphate entirely from its proposed fertilizer program. Yet, it is extremely doubtful that the figures obtained could be reliably considered as actual quantitative data and evaluated as such when planning the amount of P_2O_5 one would need to supply in the fertilizer to make up for the deficiency which apparently exists in the soil.

The nitrogen picture illustrates still further variations which are probably the effect of changes in the activity of soil organisms and the fate of their products. The nitrate-nitrogen status after the first few months decreased to a figure close to 10 pounds per acre, from which it seldom varied thereafter. The ammonia nitrogen, which was negligible for some six months at the beginning of the study, gradually accumulated thereafter. At no time, however, was the available nitrogen content sufficiently high to indicate that it would be feasible to make any allowance in the total supply that might be required and which might be proposed for a sugar cane crop.

Such evidence of variations, as we have recorded herewith, leads us to recognize better the limitations concerned with the use of soil analyses data, and indicates how hazardous it would be to evaluate the results on a strictly quantitative basis when deciding on the actual amounts of plant food to be supplied in the fertilizer for sugar cane.

TABLE I

DETAIL OF SEMI-MONTHLY SAMPLES

(All figures for nutrients are averages of two samples given as pounds per acre to depths sampled)

Date sampled	lb ammonia nitrogen 0–6″	6″–12″	12″–24″	lb nitrate nitrogen 0–6″	6″–12″	12″–24″	lb P_2O_5 0–6″	6″–12″	12″–24″	lb K_2O 0–6″	6″–12″	12″–24″	pH 0–6″	6″–12″	12″–24″	Inches rainfall (½ mo.)
July 3, 1936	—5	—5	—5	16	9	7	62	37	163	106	69	100	6.5	6.6	6.7	.99
July 18	—5	—5	—5	20	11	5	21	14	18	69	37	—75	6.5	6.6	6.7	1.76
August 3	—5	—5	—5	36	20	17	17	17	20	81	56	—75	6.4	6.6	6.7	1.70
August 18	—5	—5	—5	34	18	17	18	15	25	75	37	—75	6.3	6.5	6.5	1.73
September 3	—5	—5	—5	21	18	25	21	16	18	75	44	—75	6.4	6.5	6.6	2.33
September 18	—5	—5	—5	19	16	22	23	17	15	131	44	—75	6.6	6.7	6.9	1.64
October 3	—5	—5	—5	11	9	20	27	17	22	106	37	—75	6.5	6.5	6.7	2.02
October 18	—5	—5	—5	13	15	26	35	14	18	75	37	—75	6.6	6.6	6.7	.94
November 3	—5	—5	—5	4	7	14	31	15	23	125	33	—75	6.6	6.6	6.6	12.00
November 18	—5	—5	—5	4	7	23	..	..	..	94	44	—75	6.4	6.5	6.5	2.62
December 3	—5	—5	—5	7	10	23	27	23	28	94	44	—75	6.5	6.6	6.6	1.66
December 18	—5	—5	—5	5	8	15	39	16	28	94	44	—75	6.4	6.5	6.5	.99
January 3, 1937	—5	—5	—5	5	7	13	27	23	25	131	50	—75	6.5	6.5	6.6	.83
January 18	—5	—5	—5	4	7	15	43	23	30	150	50	—75	6.6	6.5	6.6	2.62
February 3	5	4	11	4	8	13	50	35	30	125	37	—75	6.8	6.6	6.7	8.26
February 18	5	3	5	5	9	20	50	29	62	125	33	88	6.7	6.6	6.7	7.89
March 3	6	6	7	5	7	18	50	35	25	119	33	—75	6.7	6.7	6.8	1.38
March 18	11	10	18	2	4	10	50	31	30	119	33	—75	6.8	6.8	6.9	2.71
April 3	11	9	13	3	6	10	62	19	25	112	37	—75	6.8	6.8	6.9	3.45
April 17	7	4	8	5	5	12	47	31	32	106	33	—75	6.8	6.8	6.8	.50
May 3	16	11	18	6	9	12	50	39	62	87	44	—75	6.7	6.7	6.7	9.75
May 18	10	8	12	4	4	5	50	31	82	63	37	—75	6.7	6.7	6.7	2.99
June 3	3	3	5	3	4	7	47	21	25	81	33	—75	6.6	6.6	6.6	.77
June 18	7	4	9	9	5	9	39	50	30	131	37	—75	6.6	6.5	6.5	1.19
July 3	11	7	15	6	7	8	35	29	62	62	36	61	6.6	6.6	6.6	.70
July 18	9	8	14	8	6	8	35	28	58	87	36	54	6.6	6.6	6.6	1.61
August 3	6	6	12	5	5	9	29	25	58	87	36	55	6.6	6.6	6.6	3.27
August 18	12	9	15	6	6	8	47	20	34	62	36	61	6.6	6.6	6.6	1.77
September 3	10	8	14	5	5	8	35	19	28	56	37	41	6.6	6.6	6.6	1.77
September 17	27	16	30	4	3	7	50	43	98	81	44	62	6.9	7.0	6.9	1.34
October 4	12	9	13	5	5	7	57	47	93	94	44	58	6.8	6.9	6.8	2.47
October 18	6	3	8	6	5	8	49	50	93	62	39	55	6.7	6.8	6.7	1.16
November 3	14	9	22	4	0	0	47	43	113	75	39	75	6.9	7.0	6.8	5.16
November 18	7	6	14	3	4	0	50	50	105	87	45	71	6.8	6.9	6.7	2.45
December 3	12	6	12	4	6	12	47	47	85	62	40	67	6.8	6.9	6.8	1.34
December 18	15	12	14	3	10	16	48	47	80	82	44	75	6.5	6.5	6.6	5.54
January 3, 1938	9	4	9	4	5	8	40	37	69	50	37	72	6.5	6.7	6.8	8.20
January18	6	6	7	3	4	7	35	43	62	37	38	68	6.6	6.8	6.9	.84
February 3	18	11	19	10	..	..	57	39	62	100	50	88	6.6	6.6	6.6	7.46
February 18	21	24	24	3	12	8	53	39	54	94	50	75	6.6	6.6	6.6	3.39
March 3	22	22	45	6	4	8	50	39	93	75	42	88	6.5	6.6	6.6	1.31
March 18	17	15	20	3	6	11	47	29	69	56	40	68	6.6	6.6	6.6	3.04
April 2	34	16	29	3	3	5	57	50	93	94	45	80	6.6	6.6	6.6	7.07
April 18	14	9	35	7	6	12	47	41	93	69	42	65	6.5	6.6	6.6	5.24
May 5	27	26	30	6	7	10	39	31	93	62	36	54	6.5	6.6	6.6	1.94
May 18	24	27	34	7	5	9	39	23	78	100	44	78	6.6	6.6	6.6	3.34
June 3	16	14	27	6	7	8	47	35	62	69	52	78	6.4	6.5	6.5	8.71
June 18	12	9	13	8	7	12	43	23	58	62	39	54	6.5	6.6	6.5	2.08

Note: lb N, P_2O_5, K_2O — for 0–6″, and 6″–12″ depths $= \frac{\text{p.p.m.} \times 2.5}{2}$

for 12″–24″ = p.p.m. x 2.5.

Colorimetric Method for the Determination of Sulfate in Cane Juice

By Paul E. Chu and Francis E. Hance

Colorimetric methods for the determination of sulfate have been described by a few workers. These have been principally in the field of biological chemistry.

Kahn and Leiboff (6), Wakefield (10) and others isolated sulfate as benzidine sulfate. The benzidine is diazotized and the color developed with phenol in an alkaline solution.

Hubbard (5) treated solutions of benzidine sulfate with hydrogen peroxide and ferric chloride to produce yellow solutions. These were compared with standards similarly treated.

Letonoff (8) and his colleagues used Folin's amino acid reagent in conjunction with benzidine for sulfate in serums and urine.

These methods are somewhat lengthy and proved unsatisfactory as rapid chemical methods (3, 4). It was our purpose, also, to have permanent standards for comparison with the colors developed.

In the search for a method suitable for the colorimetric analysis of sulfate in cane juice, it was noted that a number of workers, Abrahamczik and Blumel (1), Giblin (2) and Kochor (7) had used sodium rhodizonate as an external indicator, and Mutschin and Pollak (9) had used the same salt as an internal indicator in volumetric methods for sulfate.

The proposed colorimetric method utilizes the color formed by sodium rhodizonate and the excess barium chloride which is used to precipitate the sulfate in the sample.

In brief, the method follows: 0.50 ml. of 0.01 N barium chloride solution is added to a measured portion of the juice sample which is placed in a tall vial (phosphate type). The contents of the vial are shaken for ten seconds, allowed to stand one-half minute, made up to 7.0 ml. with distilled water and thoroughly mixed. Three-fourths of a ml. of a freshly prepared 0.1 per cent aqueous solution of sodium rhodizonate is added and the contents again mixed to develop the color. The sodium rhodizonate forms a red solution with the excess barium. If there is no excess barium chloride, the solution is yellow.

A set of eight permanent inorganic standards, Plate I, has been prepared to cover the range of colors developed. The test solution is compared with the sulfate standards in front of a phosphate illuminator (3). A table gives the sulfate content in terms of parts per million sulfate for various aliquots of the sample which match each of the standard tubes.

It is necessary to follow precisely the proportions given in the method when using the standards described below. After exhaustive study, the concentrations and proportions of reacting substances given in the following detailed procedure were found to be most satisfactory.

EXPLANATION OF PLATE I

COLORIMETRIC METHOD FOR THE DETERMINATION OF SULFATE IN CANE JUICE

The sealed tubes of color standards are arranged in the order of increasing sulfate content, the lowest being at the extreme left. They are placed in a wooden rack and are numbered progressively from one to eight, the lower figure denoting lower sulfate content.

Unknown solutions in open vials are placed in the intervening spaces and the whole assembly is placed in front of a standard source of illumination for comparison. Reference is made to a suitably prepared table for analytical values. The standards are made from an inorganic salt and are permanent. Full details of preparation, standardization and evaluation appear in the text.

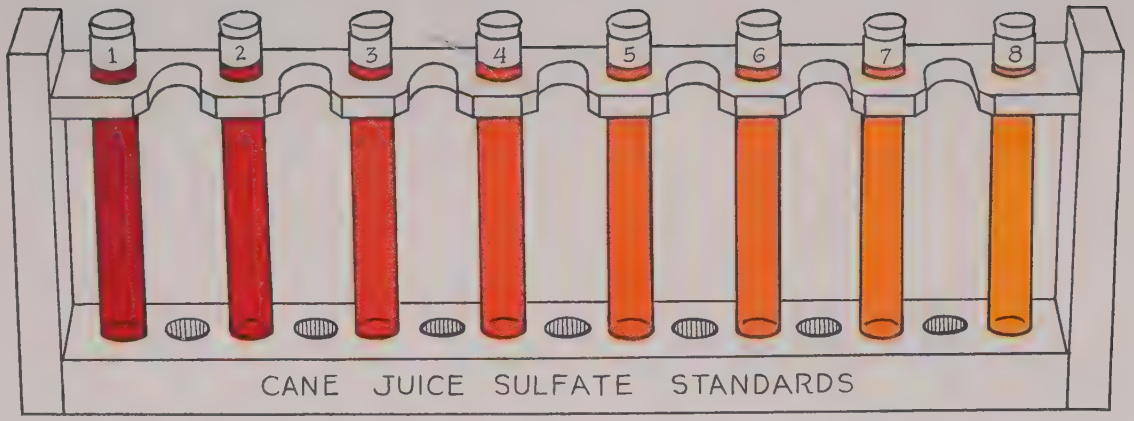

PLATE I.

Permanent Inorganic Color Standards:

The search for permanent soluble inorganic salts, or combinations of these, to match the colors developed in the test vials in the determination of sulfate encountered unusual difficulties because the red-colored barium rhodizonate was mixed with small crystals of white barium sulfate. This mixture produced a tinted turbidity instead of clear-colored solutions.

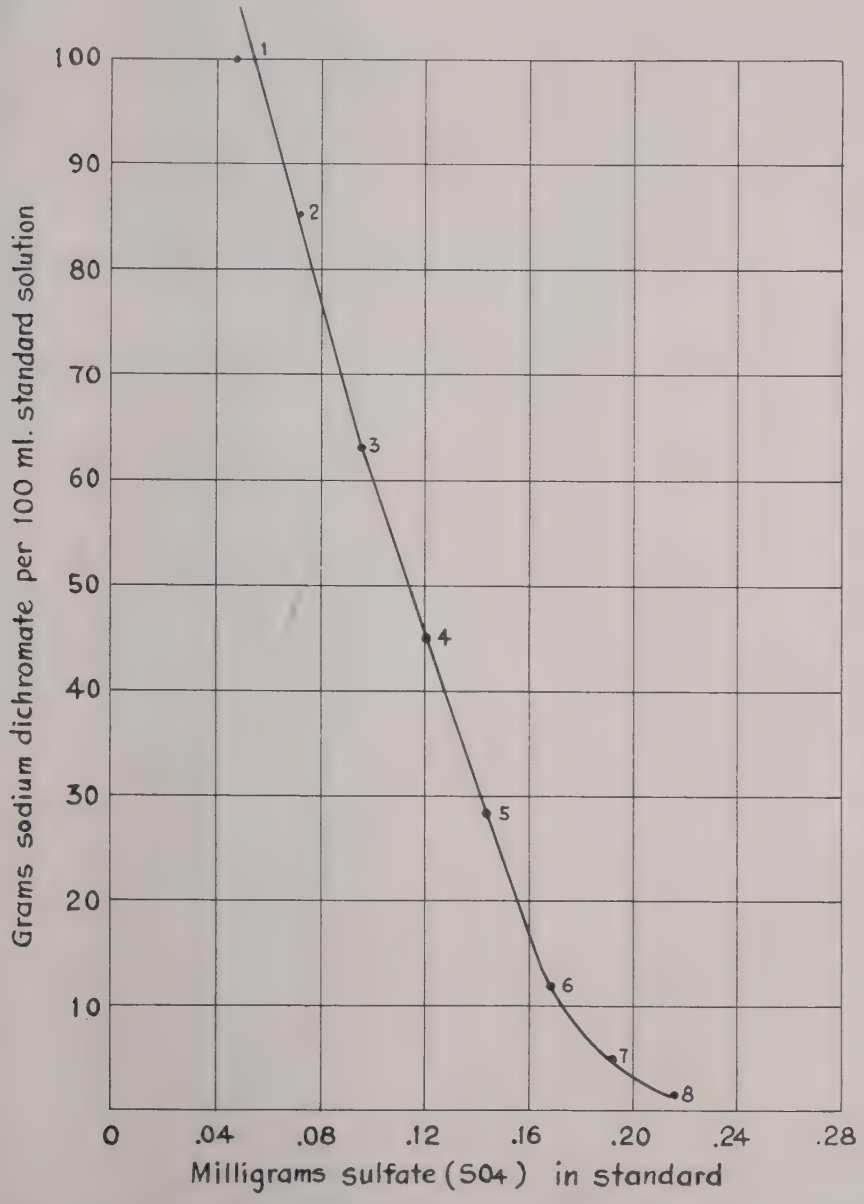

Fig. 1. Graph showing grams of sodium dichromate required to make 100 ml. of each sulfate standard and its sulfate equivalent in mg of SO_4.

The nearest approximations to the regularly developed test solutions were obtained by etching the outer surfaces of the vials in which the inorganic solutions were sealed. The etching was effected by dipping the stoppered vial, previously treated with cleaning solution, for five minutes in a proprietary etching compound ("Jack Frost").

Preparation of Standards: A concentrated aqueous solution of sodium dichromate is used as the base for the standards.

Solution A: Dissolve 250 grams C. P. sodium dichromate ($Na_2Cr_2O_7.2H_2O$) in distilled water and make up to a total volume of 250 ml. Filter.

Column 2 of Table I shows the quantity of this concentrate to use in making 100 ml. of each standard solution. Column 3 indicates the treatment given the vials in which the inorganic solutions are sealed. The sealing is effected by pouring molten paraffin into the vial filled within 20 mm. of the top with standard solution. A rubber stopper may be pushed into the opening in which case a little air space is left between the stopper and the paraffin.

TABLE I

Sulfate standard No.	ml. solution "A" per 100 ml. standard	Vial treatment	SO_4 equiv. of standard, in milligrams
1	100	Outer surface etched 5 minutes by "Jack Frost"	0.048
2	85		0.072
3	63		0.096
4	45		0.120
5	28		0.144
6	12		0.168
7	5		0.192
8	1.5	Unetched tube	0.216

Column 4 gives the sulfate equivalent in milligrams of SO_4 of each standard. In Fig. 1, the sulfate content is plotted against the concentration of sodium dichromate in the standards. The smooth curve shows the regularity of the change of color to sulfate content and also serves as a confirmation of the figures experimentally obtained.

Equipment Required:

1 set sulfate color standards in box.
6 beakers, Pyrex glass, 100-ml. capacity.
6 funnels, glass, 90-mm. diameter.
1 volumetric flask, Exax, 10-ml. capacity.
1 volumetric flask, Exax, 25-ml. capacity.
1 vial block.
1 burette, Exax, 50-ml. capacity, for distilled water.
1 box Whatman No. 12, 15-cm. folded filter paper.
1 funnel rack, 10-hole.
1 phosphate illuminator.
2 pipettes, Mohr, 1-ml. capacity, graduated to 0.01 ml.
12 vials, shell, tall form, calibrated to 7.0 ml.
1 special pipette, 0.75-ml. capacity, with rubber bulb.

Preparation of Special Equipment Required: The tall-form shell vials are calibrated by filling from a 25-ml. burette to 7.0 ml. To prepare the special pipette, draw glass tubing of 7 mm. external diameter to a tip. It is calibrated by counting the number of drops equivalent to 1 ml., then drawing up 1 ml. from a 10-ml. calibrated graduate and letting out one-fourth of the number of drops determined. Scratch a mark at the 0.75-ml. point. Enlarge the upper end to accommodate a small rubber bulb.

Reagents:

Sulfate Reagent No. 28, 0.01 N Barium Chloride: Weigh out 1.2216 grams barium chloride ($BaCl_2.2H_2O$), wash into a liter volumetric flask and make up to the mark with distilled water. Preferably, make up a liter of tenth normal barium chloride solution and dilute 100 ml. to 1 liter.

Sulfate Reagent No. 29, 0.1 Per Cent Aqueous Solution of Sodium Rhodizonate: This reagent must be freshly prepared. It loses strength gradually and can be used for only a few hours. Portions of the salt are weighed out into small glass tubes. Take the amount needed and wash completely with distilled water into the size volumetric flask indicated. Make up to volume, stopper and shake until dissolved. Three-fourths ml. of the reagent added to 7.0 ml. of distilled water in the tall vial used for developing the color matches standard No. 8. If the preceding test shows a difference in the shade, the reagent must be discarded.

Procedure:

The volume of the samples tested and also of the various reagents used must be measured accurately. Since the volumes employed are exceedingly small, it is necessary to remove any liquid clinging to the outer surface of the pipettes before inserting the latter into the vials. A clean piece of filter paper is suggested for this purpose. Likewise, before the transfer, the tip of the pipette should be touched to the outer surface of the vial and after the transfer to the inner surface. This insures more accurate results.

1. Use fresh, untreated juice or juice to which has been added the preservative employed in rapid chemical methods.
2. The juice is mixed thoroughly and is filtered through Whatman No. 12, 15-cm. folded filter paper.
3. 0.20 ml. of the juice is transferred by means of a 1-ml. Mohr pipette, graduated to 0.01 ml., *to the bottom* of a tall vial (phosphate type).
4. 0.50 ml. of sulfate Reagent No. 28 is added by means of a pipette, similar to the one used above, to the bottom of the vial containing the juice; the contents are shaken for ten seconds.
5. Allow the vial to stand ½ minute.
6. Dilute with distilled water to the 7.0-ml. mark.
7. Stopper with the finger and mix by inverting three times.
8. Add 0.75 ml. of sulfate Reagent No. 29, using a specially calibrated pipette for the purpose.
9. Mix by inverting the vial three times. Let stand for 10 seconds, then compare with the aid of the phosphate illuminator against the sulfate standards.

10. If the developed solution is too red, use more juice and repeat Steps 3 through 9. If the solution is too yellow, use less juice and repeat Steps 3 through 9.

11. Use as many aliquots as possible, the colors of which fall within the range of the sulfate standards.

12. Record all the readings. Refer to Table II which gives the sulfate concentration in terms of parts per million for various aliquots used.

13. For the final result, take the average of the figures for the aliquots matching Standard No. 3 to Standard No. 8, inclusive.

Comparison of Results:

The method has been applied to a number of representative cane juices secured in visits made to all of the plantations on Oahu. Both fresh and preserved juices were analyzed by the colorimetric method described and also by the regular gravimetric method. The results are shown in Table III.

TABLE II

COLORIMETRIC DETERMINATION OF SULFATE IN CANE JUICE
SULFATES (SO_4=) IN PARTS PER MILLION

Standard No.	ml. sample used														
	.05	.10	.15	.20	.25	.30	.35	.40	.45	.50	.60	.70	.80	.90	1.00
1	960	480	320	240	192	160	137	120	107	96	80	69	60	53	48
2	1440	720	480	360	288	240	206	180	160	144	120	103	90	80	72
3	1920	960	640	480	384	320	274	240	213	192	160	137	120	107	96
4	2400	1200	800	600	480	400	343	300	266	240	200	171	150	133	120
5	2880	1440	960	720	576	480	411	360	320	288	240	206	180	160	144
6	3360	1680	1120	840	672	560	480	420	374	336	280	240	210	187	168
7	3840	1920	1280	960	768	640	548	480	426	384	320	274	240	213	192
8	4320	2160	1440	1080	864	720	617	540	480	432	360	309	270	240	216

TABLE III

Juice No.	Plantation	Variety	Treatment of sample	p.p.m. sulfate (SO_4=) Gravimetric	Colorimetric
1	Honolulu Pltn. Co.	31-2538	Fresh	1370	1450
2	Honolulu Pltn. Co.	31-2538	R.C.M. preservative	1350	1410
3	Oahu Sugar Co., Ltd.	H 109*	Fresh	868	886
4	Oahu Sugar Co., Ltd.	H 109*	Fresh	1026	1000
5	Oahu Sugar Co., Ltd.	28-3540	Preserved	576	592
6	Ewa Plantation Co.	H 109	Fresh	1112	1320
7	Ewa Plantation Co.	H 109	Preserved	1142	1200
8	Kahuku Plantation Co.	H 109	Fresh	1064	1220
9	Kahuku Plantation Co.	H 109	Preserved	1066	1160
10	Waianae Company	H 109	Fresh	1020	1160
11	Waianae Company	H 109	Preserved	1014	1130
12	Waialua Agr. Co., Ltd.	H 109	Fresh	822	910
13	Waialua Agr. Co., Ltd.	H 109	Preserved	834	906
14	Waialua Agr. Co., Ltd.	H 109	Fresh	710	735
15	Waialua Agr. Co., Ltd.	H 109	Preserved	756	705

* Tops included.

It will be noted that although the colorimetric results vary somewhat from the gravimetric figures, the variation is within the limits of the change from one standard to the next.

Summary

A colorimetric method for the determination of sulfate in cane juice is described, including complete details of technic, instructions for making permanent inorganic standards and comparisons with gravimetric results. The method is rapid and can be carried out by trained workers.

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The Third Study of Water and Cane Ripening

By Constance E. Hartt

The first and second reports of an investigation of the importance of water in the ripening of cane were published in 1934 (1) and 1936 (4). The plants used in the third study were the ratoons of those used in the second study. A brief account of the results obtained in the third study has already been rendered (6). All of the experiments illustrate the importance of water in increasing the amount of sugar produced by the leaves, in facilitating the transport of sugar from the leaves to the stems, and in aiding the expression of sugar in the juice.

Methods

Sugar cane plants of the variety H 109, planted in pots of good soil at the Experiment Station on October 8, 1934, were ratooned on November 30, 1935, and given complete fertilization. The plants were uniformly watered twice daily for eleven months. Their growth was excellent and uniform, showing no residual effect of treatment.

On September 30, 1936, the following five series were inaugurated, with 14 pots per series:

1. Dark wet
2. Dark dry
3. Light wet
4. Light dry
5. Outdoor control

The plants in each series were taken from all parts of the plot. No attempt was made to use the root systems and ratoons therefrom of a given series in the second experiment for the same series in the third experiment.

On Wednesday, September 30, the plants of series 1-4 were placed in the greenhouse. The plants of all series were watered twice daily until Thursday noon, October 8, after which time the plants of series 2 and 4 received no more water.

Growth measurements of all plants were made twice daily from October 4 to 10 inclusive, and a final measurement on October 12. The measurements were made by Frederick F. Hébert and William O. Smith. The measurement used was the distance to the highest emerged dewlap from a constant base reference mark. Two representative stalks in each pot in each series were measured at 8 a. m. and 2 p. m. The results for each series were averaged and plotted. The graphs were used as a basis for deciding when to continue the experiment, since it has been shown by Wadsworth (9, 10) that the growth curve for sugar cane flattens out when the soil in which the cane is grown reaches the wilting point. Such a flattening of the curves following the withdrawal of water is shown in Figs. 2 and 4. The growth of the plants of series 2 and 4 had ceased by October 10, and their leaves were rolled and dried at the tips, whereas the plants of series 1, 3 and 5 continued to grow and their leaves continued to be turgid.

The plants of series 1-4 were placed in the darkened assembly room on October 10, being moved from 2-4:15 p. m. The plants of series 3 and 4 were returned to the greenhouse on October 11, being moved from 6:30-7:45 p. m. These plants received light from 6:15 a. m. until 1 p. m. October 12, when they were sampled. The plants of series 1, 3 and 5 were watered twice daily throughout the experiment.

The plants of series 1 and 2 were sampled at 8 a. m., October 12, while still in the darkened assembly room, with the aid of flashlights. The plants of series 3, 4 and 5 were sampled at 1 p. m. the same day.

In sampling, two stalks were taken from each pot, care being exercised not to select the stalks reserved for growth measurements. Counting the leaf with the highest emerged dewlap as leaf number 1, leaves number 1 and 2 were taken. The entire green-leaf cane was used. Seven stalks of dry-leaf cane from each series were taken at random, cleaned, split lengthwise, and half used in sampling. The other complete stalks were used for juice analyses without being cleaned, the juice being expressed in a Cuba mill.

The order of sampling was as follows: first the blades, then the sheaths, then the green-leaf cane, and finally the dry-leaf cane. The series were sampled in numerical order. Samples were taken for the estimation of moisture, sugars, and enzymes.

Soil samples for the determination of moisture content were taken from series 1-4 on October 12 and 13. The plants were then discarded.

Determinations were made of simple sugars and sucrose by methods described previously (5). Determinations of the activities of the enzymes invertase, amylase, dextrinase, and maltase were made by methods already outlined (4). An improvement in technique was used in the determinations of invertase and maltase, involving the use of double controls. These double controls wipe out apparent but unreal differences in activity which are caused by variations in sugar content in the plant material used in the determination of enzyme activity.

The plan of the experiment included determinations of starch and total polysaccharides. The plant material for these determinations was ground to 100 mesh in a ball mill and stored in aluminum tins until analyzed. Unfortunately the finely ground material adsorbed aluminum from the tins, which inhibited the activity of the taka-diastase used in the analysis. Several samples were lost due to breakage of the glass bottles of the ball mill. For these reasons incomplete and unsatisfactory results were obtained for starch and polysaccharides. Therefore, we are unable to calculate the results upon the usual residual dry-weight basis, because for that method of calculation one must know the percentage of polysaccharides. The usual residual dry weight is calculated by subtracting the sum of the total sugars plus polysaccharides from the dry weight. Instead, we are reporting results upon a modified residual dry-weight basis, in which the total sugars alone are subtracted from the dry weight. The results by all three methods of calculation show the same tendencies almost without exception. The differences are intensified when calculated on the modified residual dry-weight basis. For this reason, and also because it is considered the most accurate method of calculation, the discussion of results will be based upon the modified residual dry-weight method of calculation. The results were also calculated on the water basis, i. e., grams sugar per 100 grams water, but are not so expressed here because they showed no essential dif-

ference from the other methods of calculation. Because both the sugar and the moisture percentages varied, the results on the water basis depend upon two variables and are therefore not as accurate a representation of the actual changes in sugar content as the results by the modified residual dry-weight method.

Results

The measurements made of the highest emerged dewlap of two stalks per pot were averaged for each series and are plotted in Figs. 1-5. These measurements were made for four days before water was withheld, for two days after water was withheld, and on the final day of the experiment. The straight line curves in Figs. 1, 3 and 5 are indicative of uninterrupted growth in the plants supplied with water throughout the experiment. Figs. 2 and 4 show that elongation ceased one day after watering was discontinued and there was no further increase in length during the experiment. The growth curves therefore indicate that at the time of the experiment, October 12, the day the plants were supposed to conduct photosynthesis, the soils of series 2 and 4 were at or below the wilting point and the soils of series 1, 3 and 5 were well above that point.

Conclusive evidence to that effect is afforded by the actual determinations of the soil moisture content. Wadsworth (11) reported the following results for the average moisture content of the soil in series 1-4:

Series 1: 36.2%
2: 21.0%
3: 26.7%
4: 20.2%

Assuming that the permanent wilting per cent is 25.7, series 1 and 3 were well above and series 2 and 4 were well below the wilting point. The result for series 3 is below that for series 1 because some of the pots in series 3 were not sampled until October 13, whereas all the other pots of all the other series were sampled on October 12, the day of the experiment.

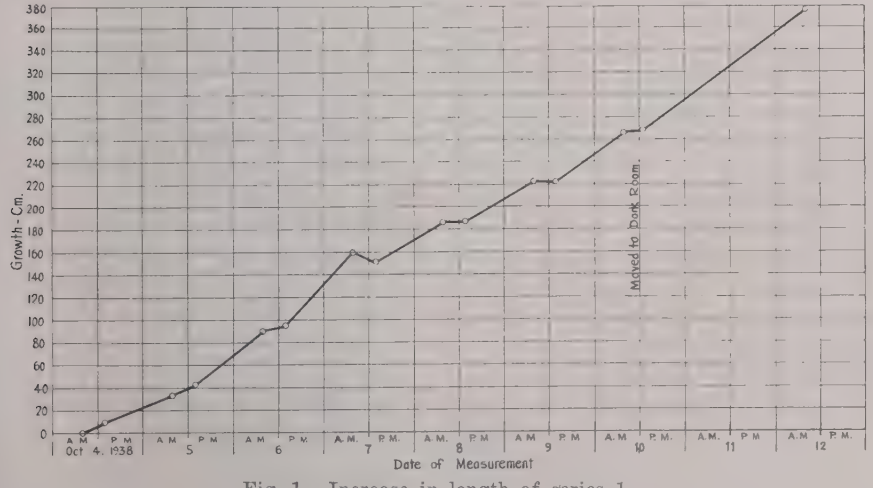

Fig. 1. Increase in length of series 1.

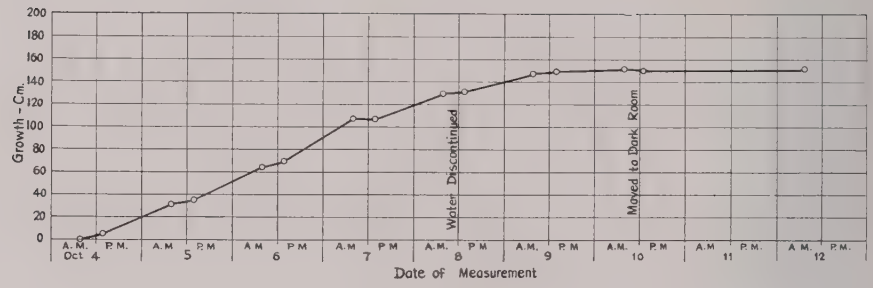

Fig. 2. Increase in length of series 2.

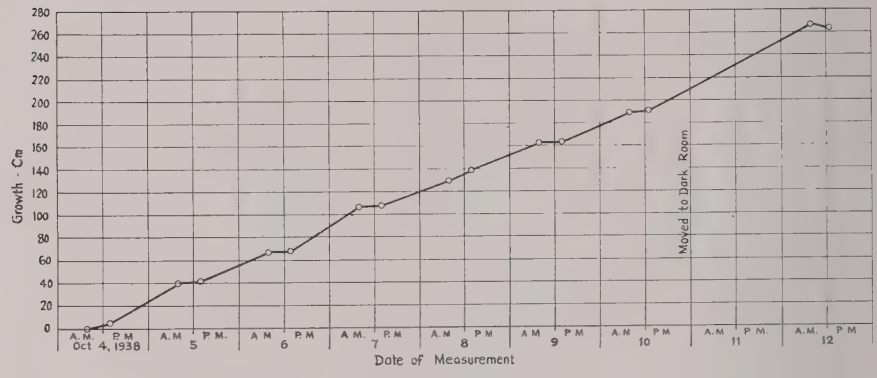

Fig. 3. Increase in length of series 3.

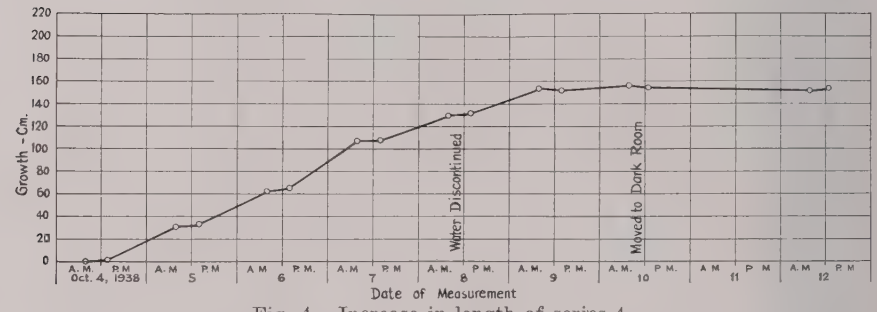

Fig. 4. Increase in length of series 4.

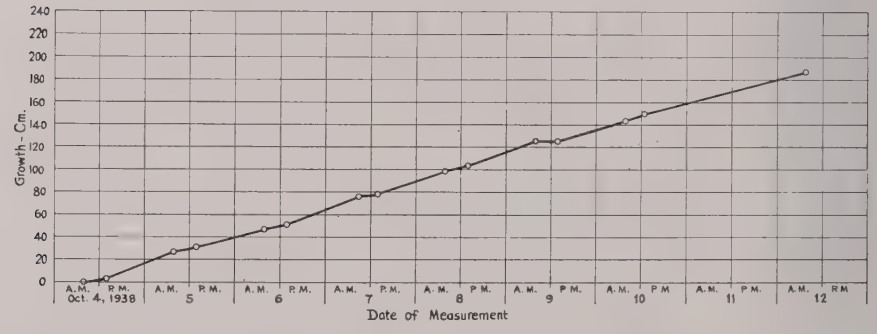

Fig. 5. Increase in length of series 5.

Fig. 6. Appearance of the plants which were watered twice daily. Photographed October 10, 1936.

Fig. 7. Appearance of the plants which had received no water for 1½ days. Photographed October 10, 1936.

Fig. 8. Appearance of the plants which had received no water for 1½ days. Photographed October 10, 1936.

The data obtained from the growth measurements and soil moisture determinations prove conclusively that we were contrasting plants in soil well above the wilting point with plants in soil at or below the wilting point, which was the object of the experiment.

The plants were photographed on October 10, 1½ days after water was withheld. The plants which were watered twice daily are illustrated in Fig. 6; those which had been deprived of water for 1½ days are illustrated in Figs. 7 and 8, and the rolling of the leaves is particularly conspicuous at the tops of the plants.

TABLE I

JUICE ANALYSES OF THE DRY-LEAF CANE

Series	Brix	Pol	Sucrose	Apparent purity	Gravity purity	Glucose	Q.R.
1. Dark wet	18.25	16.34	16.57	89.53	90.79	0.68	8.06
2. Dark dry	17.19	14.63	14.95	85.11	86.97	1.08	9.29
3. Light wet	17.68	15.38	15.64	86.99	88.46	0.96	8.71
4. Light dry	17.40	15.15	15.45	87.07	88.79	0.96	8.84
5. Outdoor control	18.98	17.25	17.50	90.89	92.20	0.56	7.56

The results of the juice analyses, which were conducted by the Sugar Technology department, are recorded in Table I, which shows that the plants of series 5 (outdoor control) had the most sucrose, the least glucose, and the best quality ratio. The plants supplied with water contained more sucrose and had a better quality ratio than the plants deprived of water, in both the dark and the light.

If the results obtained with the plants in the dark are subtracted from the results obtained with the plants in the light, the increase or decrease during the time of exposure to light is obtained. This has been done and the results are presented in Table II. Table II shows that the dry-leaf cane of the plants supplied with water deteriorated during the day in every quality tested, whereas the dry-leaf cane of the plants deprived of water improved in every category. Notwithstanding these facts, the juice of the plants supplied with water was superior to the juice of the plants deprived of water, both in the dark and in the light.

TABLE II

INCREASE OR DECREASE DURING EXPOSURE TO LIGHT FOR 7 HOURS, DRY-LEAF CANE

Series	Brix	Pol	Sucrose	Apparent purity	Gravity purity	Glucose	Q.R.
Supplied with water	—.57	—.96	—.93	—2.54	—2.33	+.28	+.65
Deprived of water	+.21	+.52	+.50	+1.96	+1.82	—.12	—.35

The results of the moisture determinations are reported in Table III, which shows that the plants supplied with water had higher moisture percentages than the plants deprived of water in all organs tested, with the exception of the green-leaf cane in the light. The blades and sheaths of the plants in the dark had higher percentages of moisture than those in the light. This difference was not found in the cane, however, in which for the most part the plants in the light had the higher percentages of moisture.

TABLE III

MOISTURE DETERMINATIONS

Series	Blades	Sheaths	Green-leaf cane	Dry-leaf cane
	Percentages expressed on wet-weight basis			
1. Dark wet	69.09±0.007	79.81±0.281	84.50±0.076	72.82±0.062
2. Dark dry	65.95±0.277	75.47±0.005	80.64±0.019	71.93±0.038
3. Light wet	64.92±0.005	76.21±0.000	81.41±0.167	74.07±0.043
4. Light dry	63.71±0.176	71.39±0.100	82.03±0.024	73.47
5. Outdoor control	64.45±0.029	74.82±0.043	81.53±0.007	71.70±0.029
	Percentages expressed on dry-weight basis			
1. Dark wet	223.6 ±0.048	394.3 ±7.680	545.4 ±3.244	268.0 ±0.859
2. Dark dry	193.5 ±1.717	307.7 ±0.048	416.6 ±0.477	256.3 ±0.525
3. Light wet	185.0 ±0.024	320.3 ±0.000	438.2 ±4.865	285.7 ±0.668
4. Light dry	175.6 ±1.336	246.4 ±2.719	456.7 ±0.811	276.9
5. Outdoor control	181.3 ±0.238	297.3 ±0.715	441.6 ±0.143	253.4 ±0.382

The results of the determinations of simple sugars are presented in Table IV and of sucrose in Table V. The increases and decreases in simple sugars and sucrose during exposure to light for seven hours, upon the modified residual dry-weight basis, are recorded in Table VI.

TABLE IV

SIMPLE SUGARS: PERCENTAGES EXPRESSED ON THE WET-WEIGHT BASIS, THE DRY-WEIGHT BASIS, AND THE MODIFIED RESIDUAL DRY-WEIGHT BASIS

Series	Wet weight	Dry weight	Modified residual dry weight
Blades:			
1. Dark wet	0.366±0.003	1.184±0.009	1.223±0.010
2. Dark dry	0.435±0.006	1.279±0.017	1.317±0.018
3. Light wet	0.303±0.001	0.866±0.004	0.914±0.004
4. Light dry	0.474±0.000	1.307±0.001	1.350±0.001
5. Outdoor control	0.349±0.001	0.984±0.003	1.028±0.003
Sheaths:			
1. Dark wet	1.669±0.001	8.268±0.005	9.537±0.006
2. Dark dry	1.552±0.010	6.327±0.042	7.076±0.048
3. Light wet	1.360	5.548	6.237
4. Light dry	1.449±0.011	5.066±0.039	5.564±0.043
5. Outdoor control	1.356±0.000	5.388±0.002	6.164±0.002
Green-leaf cane:			
1. Dark wet	2.632	16.982	28.488
2. Dark dry	2.571±0.000	13.286±0.004	22.507±0.006
3. Light wet	2.606±0.003	13.887±0.050	24.085±0.085
4. Light dry	3.047±0.000	16.957±0.002	28.459±0.004
5. Outdoor control	2.740±0.020	14.856±0.120	24.374±0.197
Dry-leaf cane:			
1. Dark wet	0.255±0.000	0.938±0.000	1.825±0.001
2. Dark dry	0.419±0.004	1.495±0.016	3.145±0.033
3. Light wet	0.687±0.001	2.651±0.006	5.165±0.012
4. Light dry	0.725±0.000	2.735±0.001	5.647±0.003
5. Outdoor control	0.417±0.002	1.476±0.007	2.830±0.013

The results of the determinations of the activity of invertase are presented in Table VII. There was no evidence that the activity of invertase was affected by treatment.

TABLE V

CANE SUGAR: PERCENTAGES EXPRESSED ON THE WET-WEIGHT BASIS, THE DRY-WEIGHT BASIS, AND THE MODIFIED RESIDUAL DRY-WEIGHT BASIS

Series	Wet weight	Dry weight	Modified residual dry weight
Blades:			
1. Dark wet	0.603±0.007	1.952±0.023	1.915±0.023
2. Dark dry	0.565±0.008	1.659±0.026	1.624±0.025
3. Light wet	1.559±0.004	4.445±0.012	4.460±0.012
4. Light dry	0.694±0.011	1.914±0.031	1.879±0.030
5. Outdoor control	1.169±0.001	3.126±0.002	3.436±0.002
Sheaths:			
1. Dark wet	1.016±0.001	5.032±0.005	5.514±0.006
2. Dark dry	1.041±0.001	4.245±0.003	4.507±0.002
3. Light wet	1.378	5.616	6.314
4. Light dry	1.112±0.009	3.887±0.033	4.055±0.035
5. Outdoor control	1.814±0.024	7.202±0.094	7.828±0.102
Green-leaf cane:			
1. Dark wet	3.625	23.407	37.304
2. Dark dry	5.359±0.000	27.681±0.003	44.545±0.005
3. Light wet	5.259	28.563	47.062
4. Light dry	4.215±0.014	23.457±0.076	37.398±0.122
5. Outdoor control	4.470±0.016	24.199±0.087	37.723±0.136
Dry-leaf cane:			
1. Dark wet	12.955±0.023	47.666±0.085	88.105±0.158
2. Dark dry	14.317	51.003	101.932
3. Light wet	11.932±0.029	46.017±0.111	85.163±0.205
4. Light dry	12.953±0.074	48.824±0.278	102.204±0.296
5. Outdoor control	13.627±0.013	48.152±0.045	90.814±0.084

TABLE VI

INCREASE OR DECREASE IN SUGARS DURING EXPOSURE TO LIGHT FOR 7 HOURS, EXPRESSED UPON THE MODIFIED RESIDUAL DRY-WEIGHT BASIS

Series	Blades	Sheaths	Green-leaf cane	Dry-leaf cane
		Simple Sugars		
Supplied with water	—.309	—3.300	—4.403	+3.340
Deprived of water	+.033	—1.512	+5.952	+2.502
		Sucrose		
Supplied with water	+2.545	+ .800	+9.758	—2.942
Deprived of water	+ .255	— .452	—7.147	+ .272

TABLE VII

INVERTASE ACTIVITY EXPRESSED IN C.C. N/20 $KMnO_4$

Series	Blades	Sheaths	Green-leaf cane	Dry-leaf cane
		Activity unbuffered		
1. Dark wet	8.36	18.36	1.30	0.00
2. Dark dry	8.40	15.78	3.23	0.00
3. Light wet	8.00	13.83	0.10	0.30
4. Light dry	7.86	15.17	2.65	0.00
5. Outdoor control	8.55	13.22	0.00	0.10
		Activity at pH 4.5		
1. Dark wet	13.75	17.64	1.24	0.00
2. Dark dry	13.25	19.48	3.97	0.00
3. Light wet	12.29	18.59	0.00	0.00
4. Light dry	14.18	18.05	1.75	0.62
5. Outdoor control	12.31	18.25	0.00	0.00

The activity of amylase was tested unbuffered and at a series of reactions ranging from pH 4.5-5.8. No activity was demonstrated in the green-leaf cane or the dry-leaf cane. The optimum reaction for amylase in blades was found to be pH 5.2, at which reaction the activity in decreasing order was: 5, 1 and 4, 2 and 3. In sheaths, the activity of amylase was very weak; there was no effect of hydrogen ion concentration and no effect of treatment.

The activity of dextrinase was determined unbuffered and at a series of reactions ranging from pH 4.6 to 8.0. No dextrinase was detected in the green-leaf cane or the dry-leaf cane. The optimum reaction for dextrinase in blades was pH 5.4-5.8; series 1 and 5 were a little more active than the other series, but they were all very weak. In sheaths, dextrinase was very weak; there was no effect of hydrogen ion concentration and no effect of treatment.

Maltase activity was very weak or absent in all four organs.

Discussion

Blades:

The blades of the plants supplied with water, as well as those deprived of water, lost water during the seven-hour period of exposure to light. This loss was only to be expected, because the moisture content of sugar cane blades grown under natural conditions reaches its lowest point in the early afternoon (2). The blades of the plants supplied with water had higher percentages of moisture than those of the plants deprived of water.

The recorded changes in simple sugars were probably insignificant.

The increase in sucrose in the blades of the plants supplied with water was ten times that in the blades of the plants deprived of water, which was barely significant.

The very small but significant increase in simple sugars and sucrose in the blades deprived of water indicated that very little photosynthesis took place in them.

These results show definitely that sugar cane requires a plentiful supply of water for the production of cane sugar in the leaves.

Sheaths:

The sheaths of both treatments lost water during the day, as do sheaths of plants grown in the open (3). The sheaths of the plants supplied with water had higher moisture percentages than those of the plants deprived of water.

The sheaths of both series lost water and lost simple sugars during the day.

The percentage of sucrose increased a little in the sheaths of the plants supplied with water, and decreased a little in the sheaths of the other plants.

Green-leaf cane:

The green-leaf cane of the plants supplied with water decreased in percentage of moisture during the day, thus resembling the blades and the sheaths. The green-leaf cane of the plants deprived of water, however, gained a little in percentage of moisture, the explanation of which is not known. A possible source of this small increase in water is the release of water bound to the complex colloids in the lower leaves attached to the green-leaf cane, many of which died during the experiment. Whatever the source, it was internal rather than external, for it is

certain that no water was supplied to the plants deprived of water during the course of the experiment.

The percentage of simple sugars decreased in the green-leaf cane of the plants supplied with water but increased in the plants deprived of water.

The percentage of sucrose increased in the plants supplied with water but decreased in the plants deprived of water. The chief cause of this difference was probably the greater supply and more rapid movement of sucrose from the blades of the plants supplied with water than from those of the plants deprived of water. Another cause was the formation of sucrose from glucose, since that process is known to take place in green-leaf cane (7, 8). The green-leaf cane of the plants supplied with water decreased in moisture content, decreased in simple sugars, and increased in sucrose, indicating that simple sugars condensed to sucrose. The green-leaf cane of the plants deprived of water increased in moisture, increased in simple sugars, and decreased in sucrose, indicating that sucrose was inverted to simple sugars. Therefore, small changes in moisture content of the green-leaf cane affect the equilibrium between the simple sugars and sucrose.

Dry-leaf cane:

The dry-leaf cane of both series gained a little moisture during the day. The percentage of simple sugars increased in both series. The percentage of sucrose decreased in the plants supplied with water, but did not change significantly.

Comparison will now be made of the results obtained on the tissue basis with those obtained by juice analysis. On the tissue basis, the dry-leaf cane of the plants deprived of water contained *more* sucrose than that of the plants supplied with water. On the juice basis, however, the dry-leaf cane of the plants deprived of water contained *less* sucrose than that of the plants supplied with water. Evidently, not all of the sucrose in the cane of the plants deprived of water was extracted by the Cuba mill. These results agree with those of the second experiment (4) in indicating that a plentiful supply of water aids in the expression of sugar in the juice.

The sucrose content of the dry-leaf cane of the plants deprived of water was not only greater than that of the plants supplied with water, but also greater than that of the outdoor control. It is evident that the increase in sucrose in the dry-leaf cane of the plants deprived of water did not occur during the day of the experiment (i. e., October 12), but rather took place during the 4-day period when water was withheld.

The results presented in Tables I-V show that the dry-leaf cane was not static. The time of day, water supply, and removal of the plants from the outside to the greenhouse are some of the factors which have been found to affect the juices. The Brix, polarization, sucrose, purity, glucose and quality ratio of the juices were affected, as well as the percentages of moisture, simple sugars, and sucrose in the tissue of the dry-leaf cane. Wadsworth (10) found a diurnal change in Brix in the dry-leaf cane of plants grown in a hot, dry environment in irrigated areas, and considered that the changes in Brix were due to diurnal changes in moisture content rather than to changes in sugar content. The results herein reported indicate that these differences are not caused solely by changes in water content but are actual differences in percentage of sugar on the dry-weight basis. Sucrose once laid down in the dry-leaf cane does not remain unchanged: it may be added

to or subtracted from. However, the fluctuations in sucrose content of the dry-leaf cane are less than those of the green-leaf cane.

Enzyme activity:

In the second study of water and cane ripening (4) it was found that the enzymes in the blades were sensitive to differences in moisture and light; the enzymes in the sheaths were less sensitive than those in the blades; maltase was the only enzyme affected in the green-leaf cane; and in the dry-leaf cane the differences were insignificant.

In the experiment reported now, there was no evidence that the activities of invertase or maltase were affected by treatment. Differences in the activity of amylase and dextrinase were small and were not consistent with those obtained in the second experiment. In short, the results of the determinations of enzyme activity presented now do not support the results obtained in the second experiment.

One cause of the differences in the enzyme results obtained in the two experiments lies in the difference in technique mentioned in "Methods." Because the double controls were used in this experiment but not in the second experiment, it is felt that the results of this experiment are more reliable than those of the second experiment. Therefore, we have as yet no evidence of differences in enzyme activity resulting from differences in moisture content due to supplying or withholding water for four days. This does not mean that drying out has no effect upon enzyme action. The leaves taken for analysis were numbers 1 and 2 (see Methods), and were not the leaves severely affected by lack of water, which were the lower leaves. In fact, the moisture percentage of the blades of the plants deprived of water did not vary greatly from that of the blades of the outdoor controls, being a little more in the dark and a little less in the light. Another study by a different method is now under way, one object of which is to find the effect of different supplies of water upon the activity of enzymes in the sugar cane plant.

Which is the better plant?

The plants supplied with water were definitely better producers of cane sugar than the plants deprived of water, for the blades of the former made ten times as much sucrose as the blades of the latter, the green-leaf cane of the former gained a large amount of sucrose while that of the latter lost sucrose, and the juice extracted from the dry-leaf cane of the former had a better quality ratio than that from the latter. It is true that on the tissue basis the dry-leaf cane of the plants deprived of water had the higher percentage of sucrose; but if the sucrose is left in the pulp when the juice is extracted in the mill, it is a loss rather than a gain. Perhaps the extraction of sugar from ripened cane requires a method different from the extraction of sugar from cane of high water content. Whether or not the addition of water to the pulp from ripened cane would result in a gain in extraction of sucrose sufficient to be of economic importance is problematical.

Summary

The third study of water and cane ripening confirms the conclusions of the first two studies: a plentiful supply of water is essential for the formation of

sucrose in the blades, for its transport to the stem, and for the expression of sugar in the juice.

Plants in soil at or below the wilting point may carry on the process of photosynthesis, but the sugar made is very much less than that made by plants adequately supplied with water. What sugar is made is stored rather than used, the result being a higher percentage of cane sugar in the dry-leaf cane of the plants deprived of water than in that of the other plants. This gain in sucrose is of physiological interest but probably not of economic importance because it is not all extracted in the juice.

Small changes in moisture content of the green-leaf cane affect the equilibrium between the simple sugars and sucrose.

The sugar content of the dry-leaf cane is not static but is affected by such factors as the time of day, the water supply, and the removal of plants from the outside to the greenhouse.

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Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD
JANUARY 3, 1939 TO MARCH 15, 1939

Date	Per pound	Per ton	Remarks
Jan. 3, 1939..	2.80¢	$56.00	Philippines.
" 5.....	2.825	56.50	Philippines, 2.80; Cubas, 2.85.
" 10.....	2.75	55.00	Puerto Ricos.
" 18.....	2.77	55.40	Philippines, Puerto Ricos.
" 20.....	2.82	56.40	Cubas.
" 24.....	2.805	56.10	Puerto Ricos, 2.80 and 2.81.
" 25.....	2.80	56.00	Puerto Ricos.
" 26.....	2.78	55.60	Puerto Ricos.
Feb. 2.....	2.775	55.50	Puerto Ricos, 2.77; Philippines, 2.78.
" 3.....	2.75	55.00	Puerto Ricos.
" 10.....	2.76	55.20	Philippines.
" 11.....	2.755	55.10	Philippines, 2.76; Puerto Ricos, 2.75.
" 14.....	2.75	55.00	Philippines.
" 15.....	2.76	55.20	Philippines.
" 20.....	2.755	55.10	Philippines, 2.75; Puerto Ricos, 2.75 and 2.76.
" 21.....	2.80	56.00	Cubas.
" 25.....	2.78	55.60	Philippines.
" 27.....	2.80	56.00	Puerto Ricos, Philippines.
Mar. 7.....	2.77	55.40	Philippines.
" 8.....	2.78	55.60	Philippines.
" 10.....	2.77	55.40	Cubas, 2.78; Philippines, 2.77; Puerto Ricos, 2.76.
" 13.....	2.78	55.60	Puerto Ricos.
" 15.....	2.80	56.00	Puerto Ricos, Philippines.

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